HYBRIDISATION AND SPECIATION IN THE GENUS *TYPHA*

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ABSTRACT

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Hybridisation is an important evolutionary mechanism with diverse outcomes, including the formation of new lineages, the exchange of alleles between species, or their extinction through genetic swamping. In some cases, hybrids exhibit higher fitness than their parental species, which can lead to hybrid invasions that threaten ecosystems. In North America, hybridisation between the native cattail *Typha latifolia* and the non-native *T. angustifolia* produces the hybrid *T.* × *glauca*, which is a highly impactful invader in wetlands across large areas of southern Canada and northern USA; contrastingly, in Europe and Asia, where its parental species also co-occur, *T.* × *glauca* is rare to non-existent. This thesis examines the evolutionary history of *T. latifolia* and *T. angustifolia*, which, in addition to being the parental species of *T.* × *glauca*, are two of the most globally widespread *Typha* species, a genus of plants foundational to wetlands. First, we developed genomic resources, including ~12M nuclear SNPs and plastome assemblies to facilitate genetic research on *Typha*; we also described a cost-effective library preparation and genotyping protocol that makes population genetic studies of freshwater plants accessible. Then, we applied those genomic resources to investigate the roles of drift, selection, and hybridisation in the divergence of *T. angustifolia*, *T. latifolia*, and their sister species, *T. domingensis* and *T. shuttleworthii*. We found that speciation in these taxa was driven by drift under allopatry, resulting from historical bottlenecks, and that natural selection has played a minimal role in the divergence of these species; additionally, we observed introgressive hybridisation from *T. latifolia* into *T. angustifolia*. Finally, we reconstructed the demographic histories of *T. angustifolia* and *T. latifolia* from North

America and Europe. We observed that reproductive isolation is strong in Europe, where the two species have potentially been sympatric for ~800,000 years, and weak in North America, where they have been sympatric for only a few centuries. Our results exemplify how the divergence and demographic histories of species can correlate with their strength of reproductive isolation. We emphasise that preventing invasions by hybrids will require limiting the movement of *Typha* and other historically allopatric species, which likely lack reproductive barriers.

Keywords: Biological invasions, cattails, demographic histories, evolutionary histories, population genomics, reproductive isolation, wetland ecosystems

PREFACE

This thesis has been written in manuscript format as all data chapters are either published or submitted to a peer-reviewed journal. Chapter 2 was published in *Freshwater Biology*, Chapter 3 is under revision for *Heredity*, and Chapter 4 is under revision for *Evolution*. The format of each data chapter is tailored to the requirements of its respective journal. For the sake of space and readability, a consistent citation format is used throughout, with a single reference section at the end of the document. The plural "we" is used in this thesis as a reflection of its collaborative nature, which means that I wrote this thesis with the guidance and collaboration of multiple people. Coauthors are indicated at the beginning of each chapter. In addition to my own papers, I contributed to the following:

Chambers, A., Chambers, B., Bhargava, D., Aleman, A., Dorken, M., and Freeland, J. (2024). A simple method to genetically differentiate invasive F1 *Typha* hybrids (*T.* × *glauca*) and advanced-generation/backcrossed hybrids from parent species (*T. latifolia* and *T. angustifolia*) in eastern Canada and northeastern USA. Journal of Great Lakes Research, 50(1), 102257. https://doi.org/10.1016/j.jglr.2023.102257

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Trent University, where this thesis was conducted, is situated on the traditional territory of the Mississauga Anishinaabeg. Mexico City, where I was born, is located on the traditional territory of the Mexica and Nahuatl peoples. Boulder, Colorado, where I will continue my scientific pursuits, is the homeland of the Apache, Arapaho, Cheyenne, Comanche, Kiowa, Pawnee, Shoshone, Sioux, and Ute. My respect and gratitude are for every one of them. ***Let everyone rise, let no one be left behind; let there not be two groups among us, we are one*** *(from the Popol Vuh, sacred book of the Mayans)*.

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NSERC supported this work. My advisors and the ENLS Graduate Program at Trent University funded me. SHARCNET and Compute Canada provided us with out-of-this-world computational resources.

Over the last four years, I earned less than the minimum wage, working both as a doctoral student and a teaching assistant. I had two extra jobs on top of that to make it through. The lack of funding and excess of work almost—literally—killed me, making me seriously consider unaliving myself and giving me anxiety attacks and shaking hands. I wish that the accumulation of those little despairs is not what makes a person an adult. Despite that, I still consider myself lucky and thankful to have done this PhD.

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CHAPTER 1: GENERAL INTRODUCTION

Throughout the evolutionary history of a species, its genetic variation is influenced by natural selection, mutations, genetic drift, and gene flow (Hedrick, 2009). These forces drive populations and species divergence, adaptations, and even extinctions (Coyne & Orr, 2004; Edwards et al., 2020). Understanding how and why the genetic variation of species fluctuates throughout their evolutionary histories and along their genomes is a central aspect of evolutionary biology, and it can help shed light on how biological diversity originates (Seehausen et al., 2014).

Genetic divergence, the formation of two or more lineages from an ancestral one, is frequently a gradual process that arises through the accumulation of genetic differences (Hartl & Clark, 2006). This process can occur under gene flow (ecological divergence) or physical isolation (allopatric divergence) (Sobel, 2016; Sobel et al., 2010). Under gene flow, differences accumulate across species' genomes when selection acts on specific traits and loci, restricting the exchange of certain alleles while the rest of the genome is admixed until gene flow is overcome (Nosil, 2012; Stankowski & Ravinet, 2021; Tigano & Friesen, 2016). In physical isolation, differences accumulate over time along species' genome as a result of changes in the allele frequencies of each lineage following mutations, drift, or natural selection (Turner & Hahn, 2010). Genetic divergence can lead to the evolution of reproductive isolation (speciation) when gene flow between lineages is inhibited (e.g., Lackey & Boughman, 2017; Ramsey et al., 2003; Richards & Ortiz-Barrientos, 2016). Genomic research allows us to describe the drivers and extent of genetic divergence and reproductive isolation among taxa, as well as how genetic variation is organised ("genetic architecture") along species' genomes (Hart et al., 2025; Hernández-Hernández et al., 2021; Nosil & Feder, 2012).

Hybridisation, the production of offspring with admixed ancestry following interbreeding between genetically differentiated taxa, is a major evolutionary mechanism that can play an important role during speciation (Abbott et al., 2013; Barton, 2008; Taylor & Larson, 2019). Hybridisation can lead to the formation of new taxa if reproductive barriers develop between parental and admixed lineages, or conversely, to the extinction of parental lineages through genetic swamping (Harrison & Larson, 2014). For instance, hybridisation between *Helianthus* sunflowers *H. annuus* × *H. petiolaris* led to the formation of *H. anomalus* (Ungerer et al., 1998), whereas in the San Francisco Bay, the native Cordgrass *Spartina foliosa* is being swamped by the invasive *S. alterniflora* (Sloop et al., 2011). In stable hybrid zones, the backcrossing of hybrids with one or both parental lineages can produce interspecific gene flow (introgressive hybridisation), a process that promotes local adaptation when introgressed alleles provide higher fitness (Pfennig, 2021; Suarez-Gonzalez, Lexer, et al., 2018; Tigano & Friesen, 2016); for example, in *Quercus variabilis* oaks, adaptive introgressions from *Q. acutissima* have driven local adaptations to novel climatic pressures (Liang et al., 2025). Together, hybrid speciation and adaptive introgressions can also result in evolutionary novelties; hybridisation of tomatoes (*Solanum lycopersicum*) and wild potatoes (*S. etuberosum*) resulted in the origin of tuberization and tuberous potatoes (*S. tuberosum*), triggering species radiation and niche expansion in the Petota lineage (Zhang et al., 2025).

Beyond its role in speciation, hybridisation is a recognised facilitator of biological invasions (Ellstrand & Schierenbeck, 2000; Mesgaran et al., 2016; Pearson, 2024). Hybrids can exhibit enhanced growth, reproductive success, or stress tolerance compared to their parental lineages (Abbott et al., 2013; Ellstrand & Schierenbeck, 2000; Schierenbeck & Ellstrand, 2009). Biological invasions by hybrid and admixed species,

many of which are caused by the anthropogenic dispersal of non-native species, have widespread negative impacts, including the displacement of native taxa (Pearson, 2024; Quilodrán et al., 2020; Todesco et al., 2016). In North America, hybridisation between two introduced saltcedars, *Tamarix chinensis* and *T. ramosissima*, produces hybrids with heterotic traits that invade riparian zones (Gaskin & Kazmer, 2009). Hybrids of the also introduced knapweeds *Centaurea stoebe* and *C. diffusa* are impactful invaders that increase soil erosion and wildfire risks across rangelands in the Great Basin (Blair & Hufbauer, 2010). Hybridisation between the introduced broomcorn *Sorghum halepense* and the native *S. bicolor* has resulted in introgressive hybridisation of several loci underlying adaptive traits, including those controlling rhizome growth, seed size, and photochemical protection, facilitating *S. halepense* invasion across the United States (Paterson et al., 2020). Understanding how hybridisation promotes invasions can inform efforts to prevent further repercussions on ecosystems, which is critical to preserving their biodiversity, functions, and resilience (Byun et al., 2023).

Wetland habitats are particularly vulnerable to invasions (Hazelton et al., 2014; Meyerson et al., 2009). These ecosystems have resource-rich conditions that favour fast-growing flora, which frequently produce extensive monotypic stands that alter habitat structure and displace native communities (Ibáñez et al., 2021; Pegg et al., 2022). Across North American wetlands, reed canary grasses (*Phalaris arundinacea*) outcompete and displace native taxa and alter water and nutrient cycles (DeBerry & Hunter, 2024). Purple Loosestrifes (*Lythrum salicaria*) form dense monocultures that reduce habitat diversity for waterfowl and invertebrates across the Great Lakes (Trebitz & Taylor, 2007). Flowering rush (*Butomus umbellatus*) and yellow flag iris (*Iris pseudacorus*) disrupt habitat structure in temperate wetlands, forming dense root mats that reduce open-water

areas, accelerate sedimentation, and diminish faunal diversity (Gallego-Tévar et al., 2022; Sandenbergh et al., 2025). The canopies of the introduced Eurasian watermilfoil (*Myriophyllum spicatum*) and its hybrid with the northern watermilfoil (*M. sibiricum*) impact North American wetlands, leading to increased sedimentation and decreased dissolved oxygen levels in these ecosystems (Glisson & Larkin, 2021). Invasive species hinder the ability of wetlands to filter water, control floods, and support wildlife, reducing the capacity of these ecosystems to provide vital ecological services (Adams et al., 2021; Pegg et al., 2022). Given that hybridisation promotes biological invasions, and because wetlands are highly vulnerable to invasive species, invasive hybrids in wetlands are a concern (Hovick et al., 2023).

Cattails (*Typha* L.) are a broadly distributed genus of rhizomatous perennial, monoecious, self-compatible, wind-pollinated, monocotyledonous flowering plants (Grace & Harrison, 1986). Cattails are foundational ecosystem engineers to wetlands (reviewed in Bansal et al., 2019), playing a vital ecological role in cycling nutrients, preventing erosion, maintaining stable water levels, and providing food and shelter for invertebrates, fish, amphibians, mammals, and birds (Bonanno & Cirelli, 2017). Additionally, cattails are a traditional and contemporary resource for agriculture, textiles, construction, and energy (Andrews & Pratt, 1978; Bidin et al., 2015; Cicek et al., 2006; Dieye et al., 2017; Kimmerer, 2013). However, some cattail species have become invasive outside their native ranges, such as *T. domingensis* Pers. in Central America and *T. latifolia* L. in Oceania (Hall, 2009; Maldonado, 2019; Z. Xu et al., 2013). Invasive cattails displace native plants, restrict water flow, reduce habitat quality, and obstruct the movement of fauna (e.g., Melvin et al., 2024; Schrank & Lishawa, 2019)—although the absolute absence of cattails would represent a greater challenge to wetlands than the

current invasions do (Trama et al., 2017). Human impacts, such as eutrophication and the introduction of exotic species, have led to increased cattail invasions in recent decades (Meyer et al., 2021).

In North America, a key driver of the invasive effects of cattails is hybridisation (reviewed in Bansal et al., 2019). *Typha* × *glauca* Godr. (*T. angustifolia* × *T. latifolia*) is a highly impactful hybrid that alters wetlands across the Great Lakes, Prairie Pothole, and Midwestern regions (Bansal et al., 2019). Invasive *T.* × *glauca* outnumbers and displaces its parental taxa (Freeland et al., 2013, 2024; Geddes et al., 2021; Pieper et al., 2020; Zapfe & Freeland, 2015), reducing biodiversity and altering freshwater ecosystems (Angeloni et al., 2006; Farrer & Goldberg, 2014; Tuchman et al., 2009), and is expanding across its already broad range across the Prairie Pothole (Joyee et al., 2024) and British Columbia (Buckholtz et al., 2025; Stewart et al., 2023). The success of *T.* × *glauca* is partially due to the high fitness of first-generation hybrids (F1s), driven by hybrid vigour, which exhibit superior growth, biomass, and competitive ability, allowing them to dominate disturbed habitats (Zapfe & Freeland, 2015). However, second-generation hybrids (F2s) experience hybrid breakdown (Bhargav et al., 2022; Pieper et al., 2017), and *T.* × *glauca* is not invasive everywhere it inhabits, like in Nova Scotia, where it neither has detrimental impacts nor outnumbers its parental species (Kirk et al., 2011).

Typha × *glauca* is scarce in Europe (Ciotir et al., 2017) and has not been reported in Asia, where its parental species also co-occur (Zhou et al., 2016), suggesting stronger reproductive isolation between *T. angustifolia* and *T. latifolia* in these continents. A possible explanation for the stronger reproductive isolation between the two species in Europe compared to North America could be selection against hybrids during different periods of sympatry. Both taxa may have been sympatric in Europe since the emergence

of the younger species, *T. latifolia*, approximately 5.7 Ma (Zhou et al., 2018). Conversely, *T. latifolia* arrived in North America through the Beringian Land Bridge from East Eurasia between 5.7 and 3.5 Ma (Zhou et al., 2018) and *T. angustifolia* was likely introduced centuries ago (Ciotir & Freeland, 2016; Freeland et al., 2013). The time in isolation that North American *T. latifolia* experienced before *T. angustifolia* was introduced may have reduced the likelihood of natural selection driving the evolution of reproductive barriers between the two species; at the same time, their extended coexistence in Europe could have facilitated this process.

Identifying why *T. angustifolia* and *T. latifolia* hybridise so successfully in North America but not in Europe or Asia could inform efforts to prevent further invasions by *T.* × *glauca* and other *Typha* hybrids. This thesis aimed to provide insights into the evolutionary histories of *T. angustifolia* and *T. latifolia* and contribute to the general understanding of hybridisation and speciation. Specifically, we aimed to i) expose how divergence under allopatry or gene flow can influence species reproductive isolation—and thus, their ability to hybridise—and ii) provide insights that help prevent future invasions by hybrid taxa. In Chapter 2, we developed a suite of genomic resources to study the population genomics and hybridisation of *Typha*. In Chapter 3, we used these resources and showed that i) the speciation of *T. angustifolia*, *T. domingensis, T. latifolia*, *T. laxmannii*, and *T. shuttleworthii* was triggered by genetic drift and occurred under allopatry as a consequence of past bottlenecks; ii) the genome-wide net distance among those five species is short despite them having diverged between ~10 and ~4 Ma; iii) most of their genomes have diverged neutrally; iv) their genomic regions under selective pressures predominantly experience balancing selection; and iv) both *T. angustifolia* and *T. domingensis* had experienced introgressive hybridisation from *T. latifolia*. In Chapter 4,

we i) reconstructed the demographic histories of *T. angustifolia* and *T. latifolia* in Europe and North America and ii) surveyed the loci that affect the patterns of reproductive isolation between the two species, exemplifying how reproductive isolation and time in sympatry could be correlated and explain why hybrids are scarce in Europe. In Chapter 5, we summarise the findings of this thesis and hypothesise how the two pathways of divergence (allopatric and ecological) could correlate to the strength of reproductive isolation between taxa.

CHAPTER 2: DEVELOPMENT OF GENOMIC RESOURCES FOR CATTAILS (*TYPHA*), A GLOBALLY IMPORTANT MACROPHYTE GENUS

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A version of this chapter was published in *Freshwater Biology*.

Abstract

A critical knowledge gap in freshwater plants research is a lack of genetic tools necessary to answer fundamental questions about their demographic histories, adaptation, and phylogenetic relationships. One example of this is *Typha*, a global genus of freshwater plants foundational to wetlands that is also becoming an increasingly problematic biological invader in numerous regions worldwide; while important insights have been discovered for this genus, existing markers are insufficient to answer fundamental questions about their demographic histories, adaptation, phylogenetic relationships, to identify introduced and hybrid lineages, and to examine patterns of hybridisation and introgression. Here, we optimised a library preparation and data processing protocol to develop genome-wide nuclear and plastid resources for studying the evolutionary history, genetic structure and diversity, hybridisation, local adaptation, invasiveness, and geographic expansion dynamics of *Typha*. We sequenced 140 samples and identified ~120K nuclear SNPs that differentiate *Typha angustifolia*, *T. domingensis*, and *T. latifolia* and retrieved their plastome sequences. We observed genetic admixture among the three species. Following a fast, straightforward, and cost-efficient genomic library preparation protocol, we produced a suite of genomic resources to facilitate investigations into the population genetics of *Typha* and to advance the understanding of wetland plants. The protocol described, the updated chromosome-level genome assembly

of *T. latifolia*, the species-specific SNPs, and the chloroplast sequences produced in this study comprise resources that can be applied to study the genetic composition of multiple populations and hybrid zones and will be incorporated into future studies of *Typha,* an ecologically important but also globally invasive macrophyte.

Keywords: Chloroplast-genome assembly, diagnostic markers, hybridisation, introgression, *Typha* phylogenomics.

Introduction

Freshwater plants are essential to aquatic ecosystems, shaping the structure and ecological functions of their habitats (Chambers et al., 2008; Christie et al., 2009; Rejmankova, 2011). Although freshwater plants have been increasingly incorporated into applications that include habitat restoration and invasive species management, they remain highly understudied compared to terrestrial plants (Evangelista et al., 2014; Iversen et al., 2022). One key knowledge gap in freshwater plants research is a lack of genetic tools necessary to answer fundamental questions about their demographic histories, adaptation, and phylogenetic relationships (Fay et al., 2019; Maréchal, 2019; O'Hare et al., 2018; Yannelli et al., 2022).

The genetic characterisation of freshwater plants has been hindered by biological and technical challenges, as well as biases in scientific research (Matheson & McGaughran, 2022; Troudet et al., 2017). Hundreds to thousands of molecular markers are often required for genomic-based research on topics such as gene flow and adaptation of non-model organisms (da Fonseca et al., 2016; Stapley et al., 2010), but the development of these resources can be time-consuming and expensive (Hu et al., 2020; Ortega et al., 2020; Prieto et al., 2021). Overcoming these challenges is now feasible using novel, rapid, and cost-effective methods that capture genome-wide genetic

variation, allowing us to address questions related to taxonomy, evolution, and conservation (Andrews et al., 2016; Goodwin et al., 2016).

Typha L. (cattails) is a global genus of rhizomatous perennial, monoecious, self-compatible, and wind-pollinated freshwater plants foundational to wetlands (reviewed in Bansal et al., 2019). Cattails are a valuable ecosystem resource and play a fundamental role by cycling nutrients, preventing erosion, maintaining water levels stable, and providing food and shelter for wildlife (Andrews & Pratt, 1978; Bonanno & Cirelli, 2017; Dieye et al., 2017; Kimmerer, 2013; Svedarsky et al., 2019). One major challenge in *Typha* research has been taxonomic identification, which cannot be fully accomplished using morphological characters due to their high intraspecific variability and interspecific hybridisation. Consequently, the richness of cattail species, their taxonomy, provenance (i.e., whether they are alien or native lineages), and phylogenetic relationships remain unresolved (Ciotir & Freeland, 2016; Volkova & Bobrov, 2022; Zhou et al., 2018). A refined *Typha* taxonomy, along with species-specific genetic markers, is necessary to identify introduced and hybrid lineages, which are increasingly documented as invasive, e.g., *T. domingensis* Pers. in Central America, *T. latifolia* L. in Oceania and Western Europe, and *T.* × *glauca* Godr. (*T. angustifolia* L. × *T. latifolia* L.) in North America (Bansal et al., 2019; Govaerts, 2004; Hall, 2009; Maldonado, 2019; Xu et al., 2013).

High-throughput sequencing technologies, novel, cost and time-accessible genome library preparations, and the assembly of the *T. latifolia* genome (287.19 Mb) (Goodwin et al., 2016; Rowan et al., 2019; Widanagama et al., 2022) collectively present an opportunity to develop a suite of genomic resources for *Typha*. In addition to taxonomic resolution, these resources will facilitate investigations of the evolutionary history, genetic structure and diversity, hybridisation and introgressions, local adaptations,

invasiveness, and geographic expansion of this genus. Here, we applied a high-throughput sequencing protocol for enzymatic fragmentation, library preparation, and data processing to produce genome-wide resources for three *Typha* spp. By optimising the method from Rowan et al. (2019), we generated a catalogue of species-specific nuclear SNPs to characterise *T. angustifolia*, *T. domingensis*, and *T. latifolia,* as well as their chloroplast genome sequences, in a fast, straightforward, and cost-efficient manner.

Materials and methods

Reference genome

We used Chromosomer 0.1.4a (Tamazian et al., 2016) to align the 1158 *T. latifolia* scaffolds from Widanagama et al. (2022) (Genbank accession: JAIOKV000000000.1) to the *T. latifolia* isolate L0001 (15 chromosomes, GenBank accession: JAAWWQ000000000.1) and produce a local chromosome-level *T. latifolia* genome. Widanagama et al. (2022) had a significantly higher mapping success of unrelated re-sequenced *Typha* spp. compared to the isolate L0001, suggesting it is a more representative genome assembly of *Typha*. The scaffolds were aligned as chromosomes using BLAST+ 2.12.0 (Camacho et al., 2009) with the software default settings, and the alignments were anchored using Chromosomer, with a gap length of 0 and a ratio threshold of 1.

Sampling, DNA extraction, and sequencing

Samples were obtained from previous studies or collected across Eurasia; DNA was extracted at Trent University following published protocols (Bhargav et al., 2022; Ciotir et al., 2017; Pieper et al., 2020, 2017; Tangen et al., 2022; Tisshaw et al., 2020). Briefly, leaf tissue was dried in desiccant silica beads and stored at -20°C. Dried leaf material was ground with a Retsch® MM300 mixer mill (Haan, Germany). DNA was

extracted from 25 to 30 mg of semi-fine powder of each sample using the EZNA Plant DNA kit (Omega BioTek) or the FastPure Plant DNA Isolation Mini Kit (Nanjing Vazyme Biotech, China) protocols for dried material, with a final elution volume of 100 µL. We obtained DNA from 38 *T. angustifolia*, 25 *T. domingensis*, and 77 *T. latifolia* samples (Figure 2.1; Supplementary Table S2.1) previously identified using a combination of genetic analyses by microsatellite loci (Kirk et al., 2011; Snow et al., 2010) and/or morphological characteristics (Grace & Harrison, 1986; Smith, 1967). Extracted DNA was quantified using a Qubit fluorometer (Thermo Fisher Scientific) and calculated as the mean of three independent readings for each sample. All samples were either standardised to 2 ng/µL by dilution with nuclease-free water or left undiluted if at concentrations less than 2 ng/µL (between 0.4 and 1.9 ng/µL).

Rowan et al. (2019) reported a relatively rapid and cost-effective library preparation technique for genomic sequencing by enzymatic fragmentation followed by ligation of short adapter sequences (i.e., tagmentation) using transposases (Nextera XT) and relatively low yields of DNA. Our protocol was based on this method with a few modifications. First, each DNA sample was tagmented with the Illumina Tagment DNA enzyme (TD) and buffer kit (small kit, #20034210). As the ratio of TD to DNA is crucial for the reaction, we initially followed Rowan et al. (2019) and subsequently optimised the reagent volumes for our library preparation as 5.5 µL of 5 × TD buffer, 0.5 µL of 1 × TD enzyme, and 4 µL of DNA (standardised or undiluted), keeping all reagents on ice during the preparation. Samples were incubated at 55°C for 10 minutes and then allowed to cool to room temperature for 5 minutes. Subsequently, 5 µL of each sample was run on an agarose gel to confirm the efficacy of the tagmentation reaction, as evidenced by the presence of visible smears. The tagmented DNA was then amplified using unique dual

indexing based on combinations from a total of 24 N7 (47 bases) and 8 S5 (51 bases) adapters (Alpha DNA, Canada). The PCR cocktail included 0.2 µM of each index, 0.5U of KAPA HiFi HotStart DNA polymerase (Roche), 12.5 µL of 5× KAPA reagent, 5 µL of DNA, and 6.5 µL of nuclease-free water, to a final volume of 25 µL. The PCR cycle consisted of 72°C (3 minutes); 95°C (30 seconds); and 14 cycles of 95°C (10 seconds), 55°C (30 seconds), and 72°C (30 seconds). Again, visible smears confirmed amplification success after running 5 µL of the PCR product on an agarose gel; then, 10 µL of each sample was pooled, and the remaining PCR products were stored at –20°C. The pooled library was purified using a QIAquick PCR purification kit (QIAGEN) according to the manufacturer's protocol, with a final elution in 50 µL of elution buffer. The library was quantified using a D1000 Tapestation assay (Agilent Technologies, USA) and a Qubit fluorometer (Thermo Fisher Scientific). A quality-control paired-end sequencing was executed using a MiSeq (151 bp) to ensure the genomic library was compiled successfully. Finally, paired-end sequencing was performed on a Novaseq 6000 (126 bp) at The Centre for Applied Genomics (Toronto, Ontario).

Raw data processing, filtering, and SNP-calling

The quality of the demultiplexed raw sequences was evaluated with FastQC 0.11.9 (Andrews, 2017) and MultiQC 1.14 (Ewels et al., 2016). Read pairing and adapter pruning were carried out using trimmomatic 0.39 (Bolger et al., 2014), removing any cleaned reads shorter than 100 bp. Paired and remaining unpaired reads were mapped to the chromosome-level *T. latifolia* nuclear genome plus the *T. latifolia* plastome reference (Genbank accession: NC_013823.1) using BWA 0.7.17 (Li & Durbin, 2009). Mapped reads from Miseq and Novaseq 6000 sequencers were merged, and mapping statistics were evaluated using SAMtools 1.15.1 (Li et al., 2009).

Genotype-calling was performed using ANGSD 0.93 (Korneliussen et al., 2014) following the SAMtools model, retrieving SNPs with a minimum *p*-value of $1e^{-6}$, minimum mapping and sequencing qualities of 20, discarding indels and triallelic sites, and outputting a binary Variant Call Format file (*-doGeno 4 -gl 1 -skipTriallelic 1 -SNP_pval 1e-6 -minMapQ 20 -minQ 20 -doMajorMinor 1 -domaf 1 -doPost 1* - doBcf 1).

For the nuclear analyses, SNPs with more than 50% missing data across all samples and the sites mapped to the plastome were removed using VCFtools 0.1.16 (Danecek et al., 2011). We did not apply any other filters to SNP identification since our samples represent a broad geographical sampling (Figure 2.1; Supplementary Table S2.1) and thus were not expected to be in Hardy–Weinberg equilibrium; additionally, as allele frequencies were unlikely to be representative of regional allele frequencies, we did not apply a minor allele frequency filter; neither did we filter for linkage equilibrium, as eliminating alleles that are in linkage disequilibrium is likely to decrease the resolution to detect hybridisation and introgressions (Alexander, 2020; Pearman et al., 2022).

Genetic structure and diagnostic markers

We used nuclear SNPs to assess the most likely number of genetic clusters across all samples and the membership of each plant to these clusters using three complementary approaches: i) ADMIXTURE 1.3.0 (Alexander & Lange, 2011) was run with *K* from 1 to 10, and the optimal number of clusters was chosen via the cross-validation procedure, ii) a neighbour-joining tree from the samples' pairwise genetic distance matrix (expressed as allele counts, transformed using the R 4.2.1 package ape 5.7-1 (Paradis et al., 2004; R Core Team, 2022)), and iii) a Principal Component Analysis (PCA), performed using Plink 1.90 (Purcell et al., 2007). To avoid over- or underestimating genetic structure (Janes et al., 2017), we verified that the assignment of samples to genetic clusters

(corresponding to three species, as described in the *Results*) was consistent across each approach.

Genetic admixture was assessed by running ADMIXTURE (K = 1–5) on three datasets, each comprising a combination of two genetic clusters, using only those SNPs that remained variable between the two species being compared. We confirmed that the cross-validation procedure for the runs of each species pair chose the optimal number of clusters as two (K = 2) and used the *Q score* as an index of admixture proportion in each sample. Applying Senn & Pemberton (2009) and Smith et al. (2018) thresholds, individuals whose Q score was $0.05 \leq Q \leq 0.95$ were considered genetically admixed.

To compare the levels of differentiation between clusters, values of relative (F_{ST}; Weir & Cockerham, 1984) and absolute genetic differentiation (d_{xy}; Nei & Miller, 1990) for every variable site were computed in 10 kb windows between species using pixy 1.2.7 (Korunes & Samuk, 2021), and the means were calculated. Species-specific SNPs were identified i) for each species' pair and ii) by running three paired comparisons of one species versus the other two on each run, using DiagnoSNPs 1.0 (Arce-Valdés, 2022). We removed genetically admixed individuals before estimating the levels of genetic differentiation and identifying species-specific SNPs.

Chloroplast genome reconstruction and phylogenetic analysis

We implemented a reference-guided workflow to reconstruct plastome sequences. Nucleotide calling was performed individually for each of the 140 samples using ANGSD, with the reads that mapped to the plastome reference, requiring a minimum mapping quality of 20 and a minimum base quality of 20, and utilising Ns for missing data (*-dofasta 2 -minMapQ 20 -minQ 20 -doCounts 1*). The sequences were aligned to the plastomes of *T. lugdunensis* P. Chabert, *T. orientalis* C. Presl, *T. przewalskii* Skvortsov,

and *Sparganium natans* (GenBank accessions: NC_061353.1, NC_050678.1, NC_061354.1, and NC_058577.1), following Smith et al. (2021) by applying MAFFT 7.0 default settings (Katoh et al., 2019). Snp-sites 2.5.1 (Page et al., 2016) was used to remove regions of the plastome with ambiguous positions, gaps, and missing data, such that if any of those were found in a sequence, that position was removed in all sequences. Nucleotide diversity (π) was calculated with the R package pegas (Paradis, 2010).

The phylogenetic relationships of the plastome sequences were reconstructed using RAxML NG 1.1 (Kozlov et al., 2019). Model selection was based on the results of jmodeltest 2.1.10 (Darriba et al., 2012), using the Akaike Information Criterion. RAxML was run under a TVM + G4 + I model with the automatic and thorough bootstrap options, starting from 100 random trees and employing *S. natans* as outgroup.

Results

Genome scaffolding, mapping statistics, and genotyping

Approximately 99.81% of the scaffold sequences were aligned to the template genome. Scaffolds were anchored to 15 chromosomes, producing a genome of 285.11 Mb (GenBank accession: JAIOKV000000000.2). The total assembled size was comparable to that of the *T. latifolia* genome sizes of Widanagama et al. (2022) (287.19 Mb) and the isolate L0001 (214.13 Mb). Updating this chromosome-level genome assembly simplified our downstream analyses while maintaining the highest mapping success for unrelated re-sequenced *Typha* spp. and facilitating future studies of speciation, hybridisation, and the genomic landscapes in *Typha*.

After quality control, 982 M clean paired-end reads were retained, and ~98% mapped to the reference genome. With minimum mapping and sequencing qualities of 20, the average depth and breadth of coverage for the nuclear sequences were 4× and 42%,

respectively. Over 60% of the plastome breadth was covered across all samples (mean depth = 711×), enabling us to use 96,591 bp for the phylogenetic reconstruction. We assembled 12,177,703 biallelic nuclear SNPs across the 140 *Typha* samples (7,122,151 with a MAF > 0.05). The total genotyping rate, i.e., the mean proportion of samples with data for each SNP, was 0.68.

Genetic structure and diagnostic markers

The ADMIXTURE analysis, the PCA, and the neighbour-joining tree each established the most likely number of genetic clusters as three ($K = 3$) (Figure 2.1): in line with previous taxonomic identifications, 38 samples were identified as *T. angustifolia* (15 of those had *T. latifolia* admixture and 5 had *T. domingensis* and *T. latifolia* admixture); 25 as *T. domingensis* (one with *T. angustifolia* admixture, 12 with *T. latifolia* admixture, and 3 with *T. angustifolia* and *T. latifolia* admixture); and 77 samples as *T. latifolia* (one with *T. angustifolia* and *T. domingensis* admixture, and one with *T. angustifolia* admixture). Using only the 18 *T. angustifolia*, 9 *T. domingensis*, and 75 *T. latifolia* non-admixed samples, the mean pairwise interspecific F_{ST} and d_{xy} values ranged from 0.25 to 0.49 and 0.28 to 0.35, respectively, with *T. latifolia* showing the highest differentiation with both *T. angustifolia* and *T. domingensis.* We identified 119,324 nuclear species-specific SNPs by pairwise comparisons between the three species and 16,856 SNPs when one species was compared to the other two (Table 2.1).

Chloroplast genome reconstruction and phylogenetic relationships

After removing ambiguities and missing data from the chloroplast genomes, we were left with an alignment of 96,591 bp across all 140 sequences and the four references, comprising 4,916 segregating sites and a π of 0.003. The phylogenetic reconstruction was congruent with the nuclear genetic structure results (i.e., individuals were consistently

assigned to the same nuclear and plastid lineages), grouping the 143 *Typha* samples into three lineages, with *T. angustifolia* in one clade, *T. domingensis* and *T. orientalis* sharing another, and *T. latifolia* and *T. przewalskii* in a third one (Figure 2.2). All interspecific nodes were strongly supported.

Discussion

We aimed to produce a suite of genome-wide resources to facilitate investigations into the taxonomy and population genetics of *Typha* and to advance the genomic understanding of wetland plants. Following a fast, straightforward, and cost-efficient genomic library preparation protocol (Rowan et al., 2019), we sequenced 140 *Typha* samples, obtaining an average breadth of 42% of the nuclear genome, characterising 119,324 nuclear SNPs that collectively differentiate three *Typha* spp., and producing chloroplast sequences with a breadth of coverage >60% per sample. With a processing time of two hours for the library preparation and a cost below 15 USD per sample, our workflow is a rapid and cost-effective protocol that can be applied in population genomic research for investigating levels of genetic diversity and differentiation, identifying conservation units and alien taxa, and studying hybrid zones, among other purposes. Additionally, our results reveal the feasibility of reconstructing chloroplast genome sequences as a byproduct of enzymatic fragmentation for high-throughput sequencing libraries, making plastome research simpler and more affordable for species with an available reference genome.

Three genetic clusters were identified from both nuclear and chloroplast genomes, corresponding to *T. angustifolia*, *T. domingensis*, and *T. latifolia*, and genetic admixture was detected among the three species. While there are some reports of hybridisation between *T. domingensis* and either *T. angustifolia* or *T. latifolia* (Ciotir et al., 2017;

Govaerts, 2004; Smith, 1967), range-wide surveys are lacking. Future research should address the extent to which hybridisation is shaping the genetic differentiation and diversity of these three species. Furthermore, *T. domingensis* is increasingly invading regions in Nigeria (Ringim et al., 2016), Costa Rica (Trama et al., 2017), and North America, potentially expanding its range across the latter (Spencer & Vincent, 2013; Zhang et al., 2008). However, the taxonomic identity of these plants is unclear—are they hybrids, non-native lineages, native lineages responding to environmental change, or misidentified *T. angustifolia* (Bansal et al., 2019)? By characterising SNPs that differentiate *T. domingensis*, we provide a valuable resource to answer this and other questions across the evolutionary history of *Typha*.

The markers that differentiate *T. angustifolia* from *T. latifolia* will have key applications in North America, where the two species interbreed across a large area and produce an invasive hybrid (*T.* × *glauca*) that dominates wetlands, alters nutrient cycling, and reduces biodiversity across the Great Lakes Region (Bansal et al., 2019); additionally, this hybrid is expanding throughout the Prairie Pothole Region, causing native plant diversity to decrease in invaded potholes (Jones et al., 2023), and may impact essential habitat for millions of breeding and migratory waterfowl species (Tangen et al., 2022). Until now, molecular resources to characterise *T. angustifolia*, *T. latifolia*, and *T.* × *glauca* were limited to sets of relatively few individual markers that have produced important insights: RAPDs, chloroplast DNA sequences, and codominant SSR loci have contributed to exposing the sexual fertility of first-generation hybrids (F1s) (Snow et al., 2010), asymmetric hybridisation, with *T. angustifolia* being mainly the maternal parent (Ball & Freeland, 2013; Kuehn et al., 1999; Pieper et al., 2017), comparable levels of sexual and clonal reproduction in parents and F1s (Pieper et al., 2020; Travis et al., 2011), heterosis

in F1s (Bunbury-Blanchette et al., 2015; Travis et al., 2010; Zapfe & Freeland, 2015), a high frequency of F1s in natural populations (Kirk et al., 2011; Travis et al., 2010), the capability of F1s to backcross, plus partial sterility in F1s coupled with hybrid breakdown of F2s and advanced-generation hybrids (Bhargav et al., 2022; Pieper et al., 2017). However, critical inquiries remain unresolved because existing markers are insufficient to expose the prevalence of advanced-generation hybrids and backcrosses in wild populations. The expansive suite of SNPs identified in this study will facilitate investigations into the extent of hybridisation, hybrid breakdown dynamics, and adaptive introgressions across the *T.* × *glauca* hybrid zone, allowing researchers to understand the processes shaping this genus speciation and species boundaries, and to inform conservation and management strategies.

Fundamental genetic tools are essential for investigating the biology, management, and conservation of freshwater plants (O'Hare et al., 2018). The protocol described in this paper, the updated chromosome-level genome assembly of *T. latifolia*, the catalogue of species-specific SNPs, and the chloroplast sequences produced comprise permanent resources that can be applied to study the genetic composition of multiple populations and hybrid zones. Genome-wide sequencing techniques and reference-based chloroplast genome assemblies are promising tools to clarify the demographic histories, local adaptation, and taxonomy of multiple congeneric lineages (Russello et al., 2015; Straub et al., 2012), and substantial genome-wide research will allow us to tackle these and other knowledge gaps in *Typha* and other freshwater taxa.

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Figures and Tables

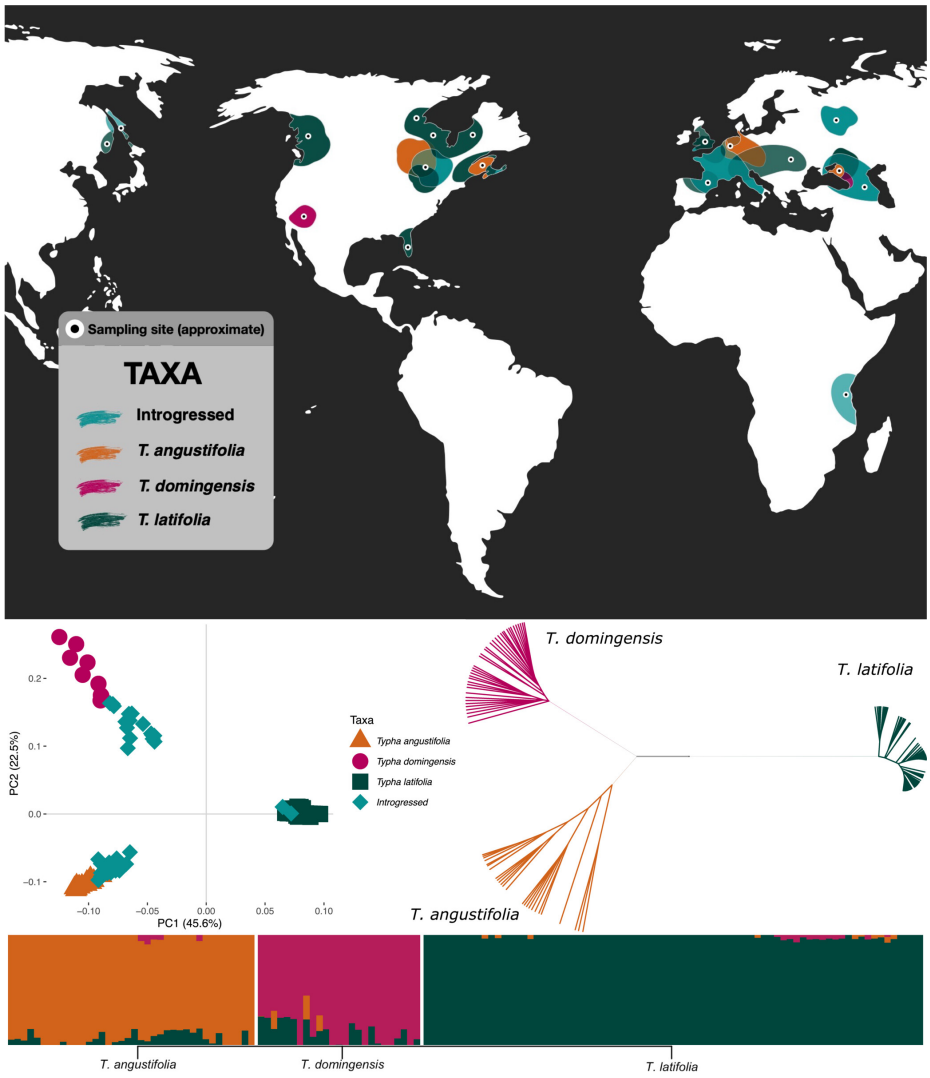

Figure 2.1. Genetic structure results from 12,177,703 nuclear SNPs obtained for three *Typha* spp. Top: Sampling locations in this study. Black points indicate approximate sampling sites, and coloured areas indicate the taxa identified. The number of samples is not shown. Left: Principal Component Analysis for PC1 and PC2. Shapes represent individuals, and colours represent taxa, as in the box. Right: Neighbour-joining tree. Branches represent individuals, and colours indicate the species to which they belong, as labelled. Bottom: ADMIXTURE (K = 3). Vertical bars represent individuals, and the admixture proportion is shown with different colours.

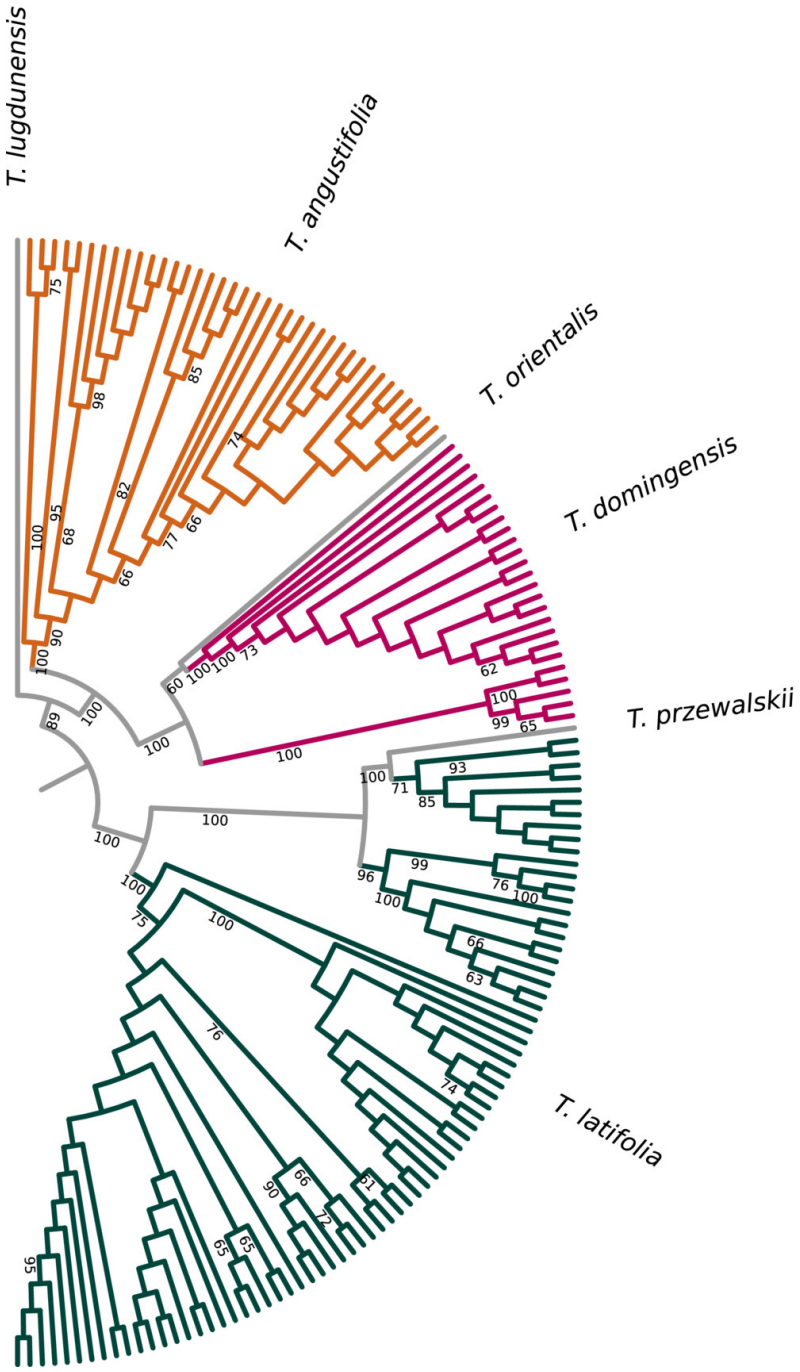

Figure 2.2. Chloroplast phylogeny of 140 samples from three *Typha* spp. and four NCBI references, based on 96,591 bp. The tree was produced with *Sparganium natans* as the outgroup and drawn without it. Branches represent individuals, and colours indicate the species to which they belong, as labelled. Numbers indicate branch support ≥ 60. Branch lengths are not shown.

Table 2.1. Mean pairwise relative (F_{ST}) and absolute genetic differentiation (d_{XY}) measured in 10 kb windows for every variable site, number of SNPs, and diagnostic markers (SNPs with fixed opposite alleles) between three *Typha* spp. When only one taxon is shown, the number of diagnostic markers represents the SNPs found when that species was compared to the other two.

Pairwise comparison	**F_{ST}**	**d_{XY}**	**SNPs**	**Diagnostic SNPs**
T. angustifolia–T. domingensis	0.25	0.28	10,358,977	33,436
T. angustifolia–T. latifolia	0.44	0.35	8,786,870	30,113
T. domingensis–T. latifolia	0.49	0.34	9,380,607	55,775
T. angustifolia	–	–	–	10,537
T. domingensis	–	–	–	3,838
T. latifolia	–	–	–	3,044

CHAPTER 3: ALLOPATRIC SPECIATION IN CATTAILS: GENOMICS REVEAL BOTTLENECKS, BALANCING SELECTION, AND INTROGRESSIVE HYBRIDISATION IN *TYPHA*, A WETLAND ECOSYSTEM ENGINEER

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A version of this chapter is under revision for *Heredity*.

Abstract

Speciation can be broadly understood within two non-mutually exclusive frameworks: genetic drift under physical isolation and natural selection under ecological divergence. Here, we examined the genomic diversity and differentiation of five *Typha* species, a group of plants that are foundational to freshwater ecosystems, with widespread, partially sympatric distributions and at least one widespread hybrid zone. Using genome-wide data from 207 individuals, we examined the contributions of demographic fluctuations, selection, and hybridisation in driving their speciation history. Demographic reconstructions revealed sequential bottlenecks and expansions that coincided with lineage splits, indicating a drift-driven speciation process with no past interspecific gene flow events for all species. Genomic landscapes were broadly neutral, exhibiting some balancing selection, sparse divergent selection, and low net divergence. Introgressive hybridisation from *T. latifolia* to *T. angustifolia* and *T. domingensis* was observed. Our findings suggest histories of allopatric divergence followed by secondary contact and contemporary hybridisation between some lineages. Our results also emphasise the roles of balancing selection and introgressions as sources of standing genetic variation. Allopatric speciation in *T. latifolia* and *T. angustifolia* could explain their ability to hybridise, highlighting the need to stop the human-mediated dispersal of *Typha* (e.g., intercontinental sourcing via garden centres).

Keywords: climate-driven barriers; divergence; drift; evolutionary history; genomic landscapes; hybridisation; secondary contact

Introduction

Speciation can follow two non-mutually exclusive pathways: physical isolation ("allopatric speciation") and natural selection ("ecological speciation") (Stankowski & Ravinet, 2021). In physical isolation, divergence is primarily driven by genetic drift (Mayr et al., 1963), and although local adaptations occur, they are not assumed to be the driving force underlying species formation (Hernández-Hernández et al., 2021). Conversely, divergent selection is mandatory during ecological speciation (Nosil, 2012; Pinho & Hey, 2010). Understanding how these pathways lead to lineages diverging is key to speciation genomics and can help us characterise how biological diversity originates (Seehausen et al., 2014).

Global climatic oscillations have repeatedly shaped the ranges and population sizes of species, triggering isolation and providing opportunities for allopatric divergence (Hewitt, 2000). These fluctuations include the rapid transformations of the Neogene (Andersson, 2009) and the glacial cycles of the Quaternary, which promoted speciation through drift events (e.g., Chacón *et al.*, 2019; Kong *et al.*, 2022; Dagallier *et al.*, 2024). Drift-driven speciation can be linked to sharp changes in species' past demographic sizes (Bock et al., 2023) and is the most common cause of divergence in plants (Hernández-Hernández et al., 2021). In contrast, natural selection promotes ecological divergence by fixing beneficial alleles and purging deleterious ones (Schluter, 2001). Under this model, speciation leads to the emergence of "genomic landscapes" (Wolf & Ellegren, 2017). These landscapes are highly differentiated ("islands") or conserved ("valleys") genomic regions along (or linked to) loci associated with natural selection and reproductive

isolation (Ravinet et al., 2017). Reconstructing these landscapes can be used to identify the contributions of selection to divergence and speciation (Andrew & Rieseberg, 2013; Han et al., 2017; Irwin et al., 2018; Ravinet et al., 2017; Shang et al., 2023).

Gene flow plays an important role in speciation (Feder et al., 2012; Tigano & Friesen, 2016). The restriction of gene flow, through allopatry or natural selection, permits genetic differentiation and the formation of distinct lineages (Morjan & Rieseberg, 2004), whereas gene flow often plays a homogenising role (Woodruff, 2001). However, gene flow between genetically distinct species (hybridisation) has diverse outcomes (Harrison & Larson, 2014; Taylor & Larson, 2019). Hybridisation can cause the formation of new species, the extinction of parental taxa through genetic swamping, or the reinforcement of species' boundaries (Garner et al., 2018; Runemark et al., 2019). In stable hybrid zones, hybridisation can cause introgressions (the movement of genes from one species to another), promoting adaptation when introgressed loci provide higher fitness (Hedrick, 2013; Suarez-Gonzalez, Lexer, et al., 2018)

Cattails (*Typha*) are rhizomatous perennial, monoecious, self-compatible, and wind-pollinated plants crucial to freshwater and brackish ecosystems; they play a vital ecological role in cycling nutrients, preventing erosion, maintaining stable water levels, and providing food and shelter for amphibians, birds, fish, insects, and mammals (reviewed in Bansal et al., 2019). The three most widespread *Typha* spp. (*T. angustifolia*, *T. domingensis*, and *T. latifolia*) have broad areas of sympatry and allopatry (Figure 3.1) and are capable of hybridising (Smith, 1967). *Typha angustifolia* and *T. latifolia* share a widespread hybrid zone in North America, involving the highly impactful *T.* × *glauca,* which forms dense stands, alters habitats, and outcompetes and displaces native plants (reviewed in Bansal et al., 2019).

Here, we examined the genomic diversity and differentiation of five cattail species to evaluate the role of drift, selection, and hybridisation in their speciation history. These comprised *T. angustifolia*, *T. domingensis*, and *T. latifolia*; we also included two closely related *Typha*, *T. laxmannii* and *T. shuttleworthii* (Zhou et al., 2018), which have more restricted ranges (Figure 3.1). The genomes of 207 plants sampled across multiple continents were sequenced to address the following questions: (1) What are the demographic histories and (2) levels of genetic diversity and divergence of these species? (3) How has selection shaped their genomic landscapes? (4) Has hybridisation led to introgressions between *T. angustifolia* and *T. latifolia*? Understanding how *Typha* species have diverged could provide important insights for wetland conservation. Speciation in allopatry (without the development of reproductive barriers) could help explain cattails' ability to create invasive hybrids; this, in turn, could highlight potential risks associated with human-mediated dispersal, such as the intercontinental sourcing of *Typha* by garden centres (Ciotir & Freeland, 2016).

Materials and Methods

Data preparation

Sampling, DNA extraction, and sequencing

Samples were obtained from a previous study (Aleman et al., 2024) and supplemented with additional collections to enhance taxonomic diversity and broaden the sampling range (Figure 3.1; Supplementary Table S3.1). Plants were pre-identified using known morphological characteristics (Grace & Harrison, 1986; Smith, 1967) and/or a combination of 3 to 4 microsatellite loci (Bhargav et al., 2022; Ciotir et al., 2013, 2017; Ciotir & Freeland, 2016; Pieper et al., 2020; Tisshaw et al., 2020). DNA was extracted following published protocols (Pieper et al., 2020; Pieper et al., 2017) and then converted

into Nextera XT libraries for reduced-representation genotyping-by-sequencing as per Aleman *et al.* (2024). Paired-end sequencing was conducted on a Miseq (151 bp) and a Novaseq 6000 (126 bp) at The Centre for Applied Genomics (Toronto, Ontario, Canada) for 207 samples (64 *T. angustifolia*, 25 *T. domingensis*, 104 *T. latifolia*, 11 *T. laxmannii*, and 3 *T. shuttleworthii*).

Raw data processing

The quality of demultiplexed raw sequences was evaluated using FastQC 0.11.9 (Andrews, 2017) and MultiQC 1.14 (Ewels et al., 2016). Read pairing and adapter pruning were carried out using Trimmomatic 0.39 (Bolger et al., 2014), removing reads shorter than 100 bp. All reads were mapped to the *T. latifolia* nuclear (15 chromosomes, 285.11 Mb, GenBank accession JAIOKV000000000.2) and chloroplast (161.57 kb, GenBank accession NC_013823) genomes with BWA 0.7.17 (Li & Durbin, 2009). Mapping statistics were evaluated using SAMtools 1.15.1 (Li et al., 2009).

Genotype-calling

Genotype calling was performed with ANGSD 0.93 (Korneliussen et al., 2014). Two datasets were created using all samples. First, SNPs were retrieved requiring minimum mapping and Phred scores of 20 and a minimum p-value of $1e^{-6}$ (referred to as the *SNP dataset*). Reconstructing the genomic landscape requires variant and invariant loci; hence, a dataset including SNPs and invariant sites (referred to as the *all-sites dataset*) was created using the same mapping and Phred filters. SNPs with more than 20% missing data and sites mapped to the plastome were removed from the *SNP dataset* in VCFtools 0.1.16 (Danecek et al., 2011). The plastome data were also removed from the *all-sites dataset*—for the coverage requirements in the *all-sites dataset*, see *Role of selection and introgressive hybridisation on species' divergence*. No individuals were

removed from any dataset. The absence of clones (multiple ramets from the same genet) was verified by calculating kinship coefficients with Plink 2.0 (Chang et al., 2015) and the *SNP dataset*.

Genetic structure and species' demographic histories

Two approaches were used to investigate the genetic structure and relationships among samples: (1) a principal component analysis (PCA; PC1 to PC3) and (2) a neighbour-joining (NJ) tree based on the samples' pairwise distances (expressed as allele counts, transformed with the R 4.3.1 package ape 5.7-1 (Paradis & Schliep, 2019; R Core Team, 2022), both produced with Plink 1.9 (Purcell et al., 2007) and the *SNP dataset*.

Species splits and migration events were inferred with TreeMix 1.13 (Pickrell & Pritchard, 2012) and the *SNP dataset*. This analysis tested migration events from 0 to 10, and the optimal number of migrations was chosen based on the data variance. Demographic fluctuations were reconstructed using a Stairway Plot 2.1.2 analysis (Liu & Fu, 2015, 2020), which generates estimates of N_e over generations in the past. Using the *SNP dataset*, a folded site frequency spectrum per species was produced in easysfs 0.0.1 (Gutenkunst et al., 2009); because we observed intraspecific structure in *T. latifolia,* distinguishing two lineages ("Eastern" and "Western", see *Results*), independent spectra were produced for each of these lineages. The resultant spectra and the mutation rate of *Arabidopsis thaliana* (7×10^{-9} mutations per site per generation (Weng et al., 2019)) were used as inputs for Stairway Plot.

Role of selection and introgressive hybridisation on species' divergence

To estimate the genetic diversity within and divergence between species, F_{ST} (Weir & Cockerham, 1984), d_{XY} (Nei & Miller, 1990), and π (Nei & Li, 1979) were computed in 5 kb windows for all species pairs with pixy 1.2.7 (Korunes & Samuk, 2021)

and the *all-sites dataset*. To ensure data reliability, windows with coverage below 50% (in each pairwise comparison) were discarded. The means for each statistic and the net divergence between species (d_a; Nei and Li (1979)) were calculated.

To test the role of selection on species' genetic differentiation, islands and valleys of divergence consistent with the types of selection proposed by Irwin *et al.* (2018) were identified using the retained windows in each pairwise comparison (see *above*). Individual F_{ST}, d_{XY}, and π values were standardised into Z-scores (e.g., ZF_{ST} = *[(window* F_{ST} *– genome-wide median* F_{ST}*) / genome-wide* F_{ST} *standard deviation]*), and windows with exceptionally high or low diversity and divergence were characterised by detecting outliers where the absolute value of a Z-score ≥ 1.96; intermediate d_{XY} values were also documented (Z-score between –0.1257 and 0.1257). Following Kessler *et al.* (2023) and Shang *et al.* (2023), islands and valleys of divergence (i.e., positive and negative F_{ST} outliers) were classified into four models of selection using joint combinations of statistics as follows: (i) divergent with gene flow (high F_{ST} and d_{XY}, low π); (ii) divergent without gene flow (high F_{ST}, intermediate d_{XY}, and low π); (iii) background (high F_{ST}, low d_{XY} and π); and (iv) balancing (low F_{ST}, high d_{XY} and π). Additionally, Tajima's D (Tajima, 1989) was estimated in 5 kb windows for each species using VCFtools and the *all-sites dataset*. Within each species, only those windows with more than 50 SNPs were kept, and loci with Tajima's D higher than +2 or lower than –2 were interpreted as experiencing balancing selection and selective sweeps, respectively.

ABBA BABA was used to identify introgressions; *T. laxmannii* was designated as the outgroup, and all possible combinations of donor and recipient species were examined (Supplementary Table S3.2). ABBABABAwindows.py (Martin & Jiggins, 2017) was used to compute f_d (Martin et al., 2015) in 5 kb windows. Two or more loci with a

positive f_d, ZF_{ST} and $Zd_{XY} \leq -1.96$, and at least within 50 kb of each other, were classified as introgressions. Introgressions from *T. latifolia* to *T. angustifolia* were observed (see *Results*); thus, ABBA BABA pattern was replicated, splitting the data into "North America" and "Europe" to test whether introgressions occur on both continents.

Results

Genetic structure and species' demographic histories

We assembled 21,759,123 nuclear SNPs across 207 samples and kept 77,207 with missing data below 20%. The PCA (PC1 to PC3, Supplementary Figure S3.1) and NJ tree (Figure 3.2) distinguished all five species; the NJ tree also revealed intraspecific structure in *T. latifolia* distinguishing two lineages, named "Western" (n = 77; North America, Western and Central Europe) and "Eastern" (n = 27; Eastern Europe and the Iturup Island in the Russian Kuril Chain).

TreeMix indicated *T. angustifolia–T. domingensis* and *T. latifolia–T. shuttleworthii* as sister lineages, and *T. laxmannii* as outgroup species (Figure 3.1); this topology was consistent with the NJ tree. The most likely number of historical gene flow events was determined to be zero, explaining over 99.9% of the data variance. *Typha laxmannii*, inferred by Treemix to be the earliest diverging species, exhibited a stable N_e from ~18 to ~5 million generations ago, until a sharp decline coincided with sharp expansions for both *T. angustifolia* and *T. domingensis*. Another sharp decline for *T. laxmannii* coincided with a sharp expansion ~3 million generations ago for Eastern *T. latifolia*. Western *T. latifolia* appeared to experience a cycle of expansion-decline-expansion between ~2 million and ~400,000 generations ago; finally, *T. shuttleworthii* appeared to have undergone a sharp expansion ~1 million generations ago. The series of sharp declines and expansions inferred by Stairway Plot overlapped with the order of species splits revealed by Treemix;

moreover, assuming a ~2-year generation time, this series of events coincides with the divergence times of the species in this study from the literature (Figure 3.3*;* see *Discussion*).

Role of selection and introgressive hybridisation on species' divergence

Covering 65% of the genome (variant and invariant sites, depth = 4×), mean F_{ST} varied from 0.317 (*T. angustifolia–T. domingensis*) to 0.617 (*T. latifolia–T. laxmannii*), d_{XY} from 0.016 (*T. latifolia–T. shuttleworthii*) to 0.030 (*T. domingensis–T. shuttleworthii*), d_a from 0.007 (*T. latifolia–T. shuttleworthii*) to 0.015 (*T. latifolia–T. angustifolia*), and π from 0.005 (*T. latifolia*) to 0.024 (*T. domingensis*), (Table 3.1; Figure 3.2). *Typha latifolia* was the only species with low genetic diversity ($\pi < 0.01$). The net divergence among the five species was low ($d_a < 0.02$).

The genomic landscapes between species pairs revealed a minor and heterogeneous role of selection in species divergence (Supplementary Figures S3.2 to S3.11). Most loci (5 kb) with exceptionally high or low F_{ST} could not be associated with any of the types of selection tested (on average, 1398 out of 1839; Table 3.2). On average, from the 441 loci that could be associated with selection, balancing selection was the most frequently detected in pairwise comparisons (375 loci, 0.66% of the genome); background selection was second (46 loci, 0.08% of the genome), followed by selection without and with gene flow (15 and 6 loci, 0.03%, and 0.01% of the genome, respectively). An island of background selection was observed between *T. laxmannii* and most species in our study (~350 kb on chromosome 14); no genes could be identified within this island.

Except for *T. latifolia*, all species had mean positive Tajima's D per species, from 0.05 (*T. angustifolia*) to 1.55 (*T. laxmannii*) (Figure 3.4) and more loci with values higher

than +2 (~8,129) vs lower than –2 (~43), consistent with the widespread balancing selection observed previously across the genomic landscapes; *T. shuttleworthii* had a mean Tajima's D of 0.66 and no loci with values higher than 2, *T. domingensis* mean Tajima's D was 1.14; and *T. latifolia* had a mean negative Tajima's D (–1.27) and a large number of windows lower than –2 (12,252), in line with the most recent expansion observed in its demographic history.

The f_d identified introgressions from *T. latifolia* to *T. angustifolia* on chromosomes 11 and 14 (~145 kb); no genes could be identified in these introgressions. The introgressed regions exhibited values of Tajima's D from –0.77 to –1.96, suggesting subtle positive selection. Splitting the data by continent confirmed that these introgressions are present in both North America and Europe (Supplementary Figures S3.12 and S3.13). Additional introgressions from *T. latifolia* to *T. domingensis* were identified in chromosomes 8, 14, and 15; no genes were found in these introgressions, and their Tajima's D values ranged from –0.004 to –1.73.

Discussion

During allopatric speciation, genetic differentiation primarily accumulates through drift (Abbott et al., 2013); in contrast, ecological (sympatric) speciation requires divergent selection (Nosil, 2012; Rundle & Nosil, 2005). This means that ecological speciation could produce stronger reproductive barriers than geographic isolation, and that speciation in isolation could enable hybridisation if species experience secondary contact (Hewitt, 2000; Rheindt & Edwards, 2011; Sobel, 2016)—although the later development of genetic barriers between species (e.g., through reinforcement (Noor, 1999)) would depend on their shared evolutionary history after secondary contact and hybridisation occur (Moran et al., 2021; Runemark et al., 2019). Here, we examined the genomic

diversity and differentiation of five cattail species to evaluate the role of drift, selection, and hybridisation in their speciation history. We revealed (1) species splits (without historical migration events) coincident with past sharp demographic declines, (2) low net genomic distance between species (d_a from 0.007 to 0.015), (3) valleys of balancing selection between species, and (4) introgressions from *T. latifolia* to *T. angustifolia*—both in North America and Europe, despite the absence of hybrids in the latter continent—and *T. domingensis*. Our results suggest that demographic contractions could be the primary factor responsible for the speciation of these *Typha*, possibly followed by range expansions and secondary contact, which enabled hybridisation between some species.

Demographic history and scarcity of divergent selection in Typha

Treemix reported a topology with no migration events, indicating that species split without interspecific gene flow can account for all the observed genetic variation among the species in our study. While it does not rule out the possibility of hybridisation after secondary contact, this result suggests that differentiation occurred in the absence of interspecific gene flow among the species in this study. Stairway Plot revealed a series of sharp N_e declines and expansions that coincide chronologically with the Treemix topology and the divergence times of Zhou *et al.* (2018). Geoclimatic events, including the Miocene aridification and the Quaternary glacial cycles (Butiseacă et al., 2021; Herbert et al., 2016; Hewitt, 2000), may have led to these bottlenecks, as both periods potentially fragmented the extent and connectivity of wetland habitats. These reductions in N_e likely reflect episodes of geographic isolation that triggered species divergence via genetic drift. Stairway Plot also indicated that *T. latifolia* possibly underwent a cycle of declines and expansions between ~2 million and ~400,000 generations ago, which is in

line with the genome-wide negative Tajima's D (–1.765) reflecting the most recent expansion in this species.

The islands and valleys of divergence associated with divergent selection were scarce, occurring on average in 0.12% of the genome and 2.88% of the loci with exceptionally high or low F_{ST} in pairwise comparisons. This result suggests that ecological divergence played a negligible role in the speciation of *Typha* in this study. Some signatures of selection-driven divergence could have been eroded by time, which is consistent with the late stages of speciation (Burri et al., 2015)—the age of *Typha* is estimated between 20 and 70 Ma (Widanagama et al., 2022; Zhou et al., 2018)—however, the net divergence (d_a) among these *Typha* species is low (<0.02; 'grey zone of speciation' (Roux et al., 2016)), which suggests that reproductive isolation and thus, speciation, remain incomplete despite *Typha* being and old genus and the high relative differentiation between species ($F_{ST} > 0.25$ (Wright, 1984)).

Balancing selection and introgressions as sources of genetic variation

Standing genetic variation can be as crucial as mutations to adaptation (Barrett & Schluter, 2008; Matuszewski et al., 2015). Several mechanisms maintain standing genetic variation, including balancing selection (Llaurens et al., 2017). Across the genomic landscapes of the species in our study, most regions associated with adaptive divergence were valleys of balancing selection, as confirmed by Tajima's D results, in which the maintenance of standing polymorphisms causes an excessively reduced divergence (Guerrero & Hahn, 2017). Signatures of balancing selection are congruent with *Typha's* ability to self-fertilise; selfing leads to increased homozygosity, so balancing selection can be crucial in preserving polymorphism at important loci (Glémin, 2021). Genes experiencing balancing selection have been shown to underlie a wide range of phenotypes

(Isildak et al., 2021; Promy et al., 2023) and promote adaptation to divergent habitats (Delph & Kelly, 2014; Wu et al., 2017). A predominant role of balancing selection has been detected among other widespread plants, including *Arabidopsis*, *Arbutus*, *Capsella*, *Populus*, and *Quercus* (Bachmann et al., 2018; Koenig et al., 2019; Le Veve et al., 2023; Meireles et al., 2017; Rendón-Anaya et al., 2019; Santiso et al., 2016; Shang et al., 2023; Wang et al., 2019; Wang et al., 2020; Wu et al., 2017). Growing evidence supports a central role for balancing selection (Delph & Kelly, 2014; Fijarczyk & Babik, 2015; Guerrero & Hahn, 2017; Kessler et al., 2023), challenging the view that background selection is the leading force shaping species' genetic diversity (Comeron, 2017).

Introgressive hybridisation is another important source of standing genetic variation (Suarez-Gonzalez, Lexer, et al., 2018; Tigano & Friesen, 2016). Research on the *T.* × *glauca* hybrid swarm in North America has demonstrated the fertility of F1 hybrids in natural populations (Kirk et al., 2011; Snow et al., 2010; Travis et al., 2010) and their ability to backcross (Bhargav et al., 2022; Pieper et al., 2017). Under these circumstances, introgressions between *T. latifolia* and *T. angustifolia* could be expected, and those identified were under positive selection. Introgressions were also present in Europe, where these species do not currently hybridise (Ciotir et al., 2017)—suggesting that hybridisation between these species may have happened in the past. Introgressions have been documented in other widespread plant species, including *Helianthus* (Kim & Rieseberg, 1999; Whitney et al., 2006, 2010), *Populus* (Rendón-Anaya et al., 2021; Suarez-Gonzalez, Hefer, et al., 2018), and *Quercus* (Goicoechea et al., 2019), and are recognised as sources of genetic variation for adaptation.

Genetic diversity in Typha

Genetic diversity reflects the reservoir of traits and potential responses to environmental changes that species encounter, thereby influencing their resilience in different ecosystems (Frankham et al., 2002; Gregorius, 1987; Hartl & Clark, 2006). Excluding *T. latifolia*, species' genetic diversity was high ($\pi > 0.01$ (Begun et al., 2007)), consistent with large population sizes and substantial gene flow. Low genetic diversity is considered detrimental, as it can reflect a low adaptive potential (Kardos et al., 2021; Teixeira & Huber, 2021). However, some taxa, including invasive species, thrive despite having low genetic diversity (Charlesworth & Jensen, 2022; Roman & Darling, 2007; Tsutsui et al., 2000). *Typha* tolerates wide-ranging climates, nutrients, pHs, pollutants, and water levels (Kadlec & Wallace, 2008; Sesin et al., 2021; Sojda & Solberg, 1993), and the most widespread and commonly recognised cattail species is *T. latifolia*, presumed to have large census sizes, and is invasive in Oceania (Xu et al., 2013). Low genetic diversity in *T. latifolia* suggests that alternative mechanisms, such as epigenetic modifications and associated phenotypic plasticity, may be responsible for the success of this species (Mounger et al., 2021). In *Arabidopsis thaliana*, epigenetic diversity underlies morphological variation and phenotypic plasticity when genetic diversity is low (Kooke et al., 2015; Schmid et al., 2018; Zhang et al., 2013). A future research inquiry is testing whether epigenetic modifications and phenotypic plasticity are more extensive in *T. latifolia* than in other *Typha* species.

Conclusions

Understanding the causes of speciation is a central aspect of evolutionary biology. Here, we tested the roles of drift, selection, and hybridisation in driving species divergence in *Typha*, an old and widespread plant genus that is foundational to freshwater

ecosystems. Our results support bottlenecks and geographic isolation as the primary causes of speciation, as well as widespread balancing selection across *Typha*, and introgressions from *T. latifolia* into *T. angustifolia* and *T. domingensis*. The absence of divergent selection does not rule out the role of ecological divergence as a promoter of speciation in *Typha,* as this genic view of speciation requires only a handful of genes (Lexer & Widmer, 2008); rather, it indicates a primary role for genetic drift. Our results add *Typha* to the body of evidence supporting an important role of balancing selection in preserving standing genetic variation despite the accumulation of divergence (e.g., Selechnik *et al.*, 2019).

Acknowledgements

We acknowledge that the laboratory procedures and data analyses were conducted at Trent University, which is on the traditional territory of the Mississauga Anishinaabeg, to whom we show our respect. We thank Polina A. Volkova and Tulsi Patel for their invaluable contributions to the laboratory and the field. The Natural Sciences and Engineering Research Council of Canada financially supported this work, and Alberto Aleman is funded by the Environmental and Life Sciences Graduate Program at Trent University. SHARCNET and Compute Canada provided computational resources for this study. We are grateful to Camille Kessler, Marie-Laurence Cossette, Beibei Zhou, Xinwei Xu, the Associate Editor, and the anonymous reviewers for their valuable feedback, which significantly improved the quality of this manuscript. Finally, we thank Enrique Ruiz for his work in Figure 3.1.

Figures and Tables

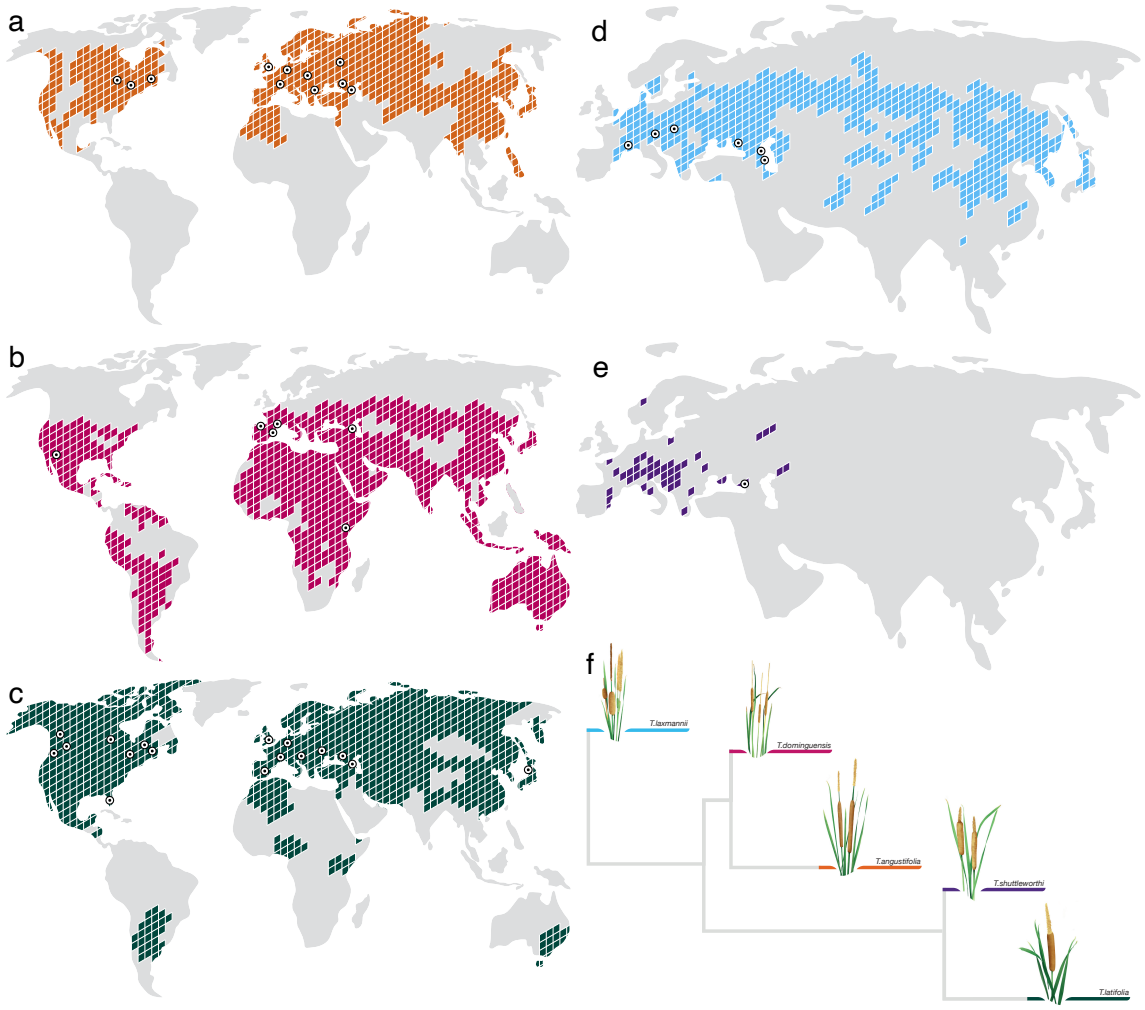

Figure 3.1. Distributions (grids) and sample sites (circles) in this study for *T. angustifolia* (a), *T. domingensis* (b), *T. latifolia* (c), *T. laxmannii* (d), and *T. shuttleworthii* (e). Distributions from Ciotir & Freeland (2016) and GBIF. Sample sizes are not represented graphically. (f) Species relationships inferred by Treemix; the maximum likelihood tree produced indicated zero migration events. The colour scheme represents species, as in the tree in (f). Note: Shading is based on maps that represent geopolitical boundaries of areas from which species have been recorded.

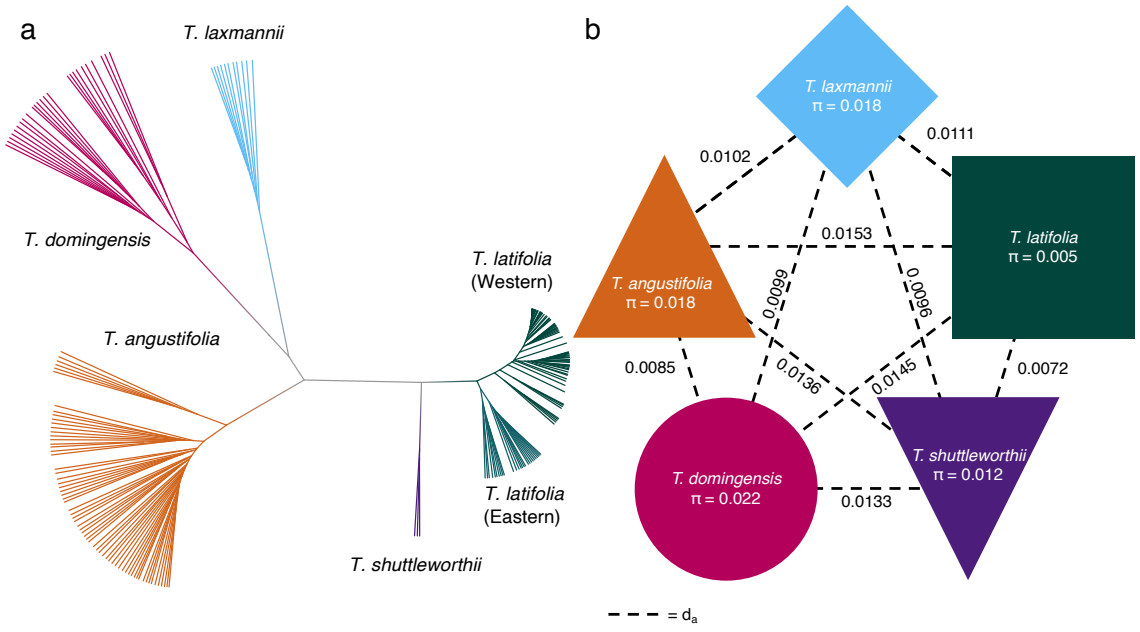

Figure 3.2. Genetic structure, species relationships, and diversity and divergence for five *Typha* species. (a) Neighbour-joining tree. Branches represent individuals; different colours indicate species, as in the labels. (b) Nucleotide diversity (π, inside shapes) for each species and net divergence (d_a, dashed lines) between species. Shapes and colours represent species.

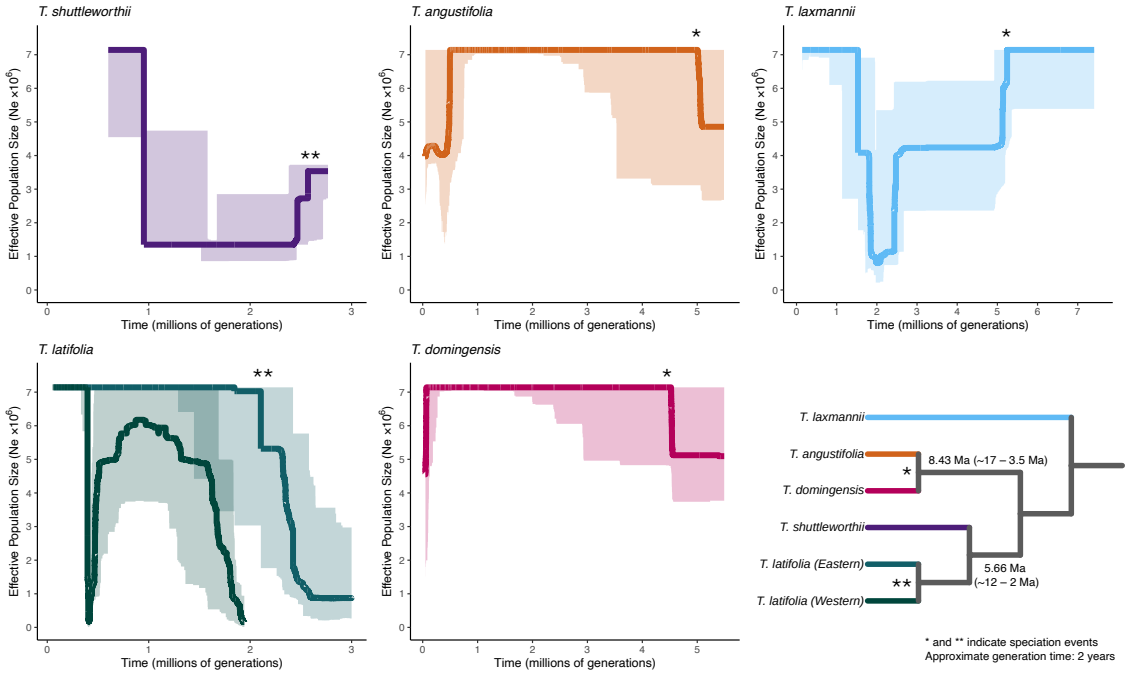

Figure 3.3. Evolutionary histories of the *Typha* in this study. Demographic fluctuations over generations in the past, reconstructed using Stairway Plot. The tree (bottom right) is based on the TreeMix results in this study; the dates correspond to Zhou *et al.* (2018); * and ** indicate speciation events.

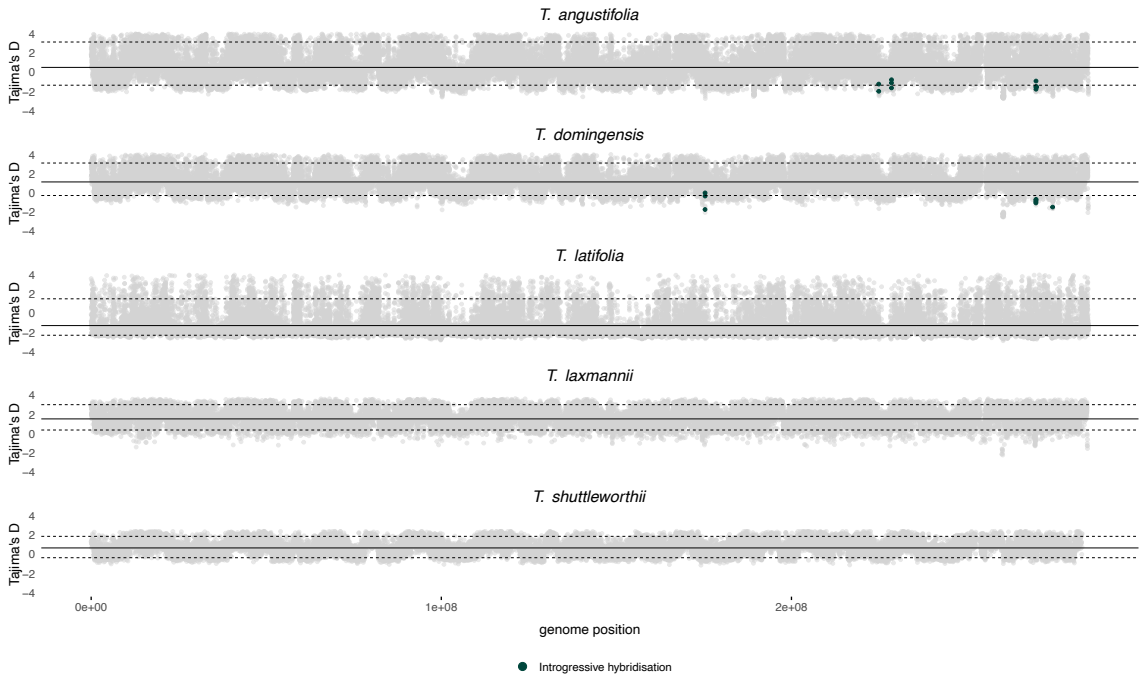

Figure 3.4. Genome-wide Tajima's D for five *Typha* species. Points represent 5 kb windows, solid lines represent means, and dashed lines represent 95% confidence intervals. Introgressions from *T. latifolia* to *T. angustifolia* and *T. domingensis* detected by ABBA BABA are highlighted in green.

Table 3.1. Mean relative (F_{ST}, below diagonal) and absolute (d_{XY}, above diagonal) genetic divergence between five *Typha* species, measured in 5 kb windows.

	T. laxmannii	*T. domingensis*	*T. angustifolia*	*T. shuttleworthii*	*T. latifolia*
T. laxmannii		0.029	0.028	0.024	0.023
T. domingensis	0.330		0.029	0.030	0.029
T. angustifolia	0.384	0.317		0.028	0.027
T. shuttleworthii	0.380	0.398	0.447		0.016
T. latifolia	0.617	0.614	0.575	0.544	

Table 3.2. Number of genetic islands and valleys of divergence (5 kb) consistent with alternative types of selection among five *Typha* species: (i) divergent with gene flow, (ii) divergent without gene flow, (iii) background selection, (iv) balancing selection. The number of islands and valleys of divergence that could not be assigned to the alternative types of selection tested is shown in (v).

	(i)	(ii)	(iii)	(iv)	(v)
T. angustifolia - T. domingensis	0	23	46	551	1384
T. angustifolia - T. latifolia	0	1	10	45	2169
T. angustifolia - T. laxmannii	0	18	82	585	996
T. angustifolia - T. shuttleworthii	0	15	99	468	1255
T. domingensis - T. latifolia	1	5	9	123	1932
T. domingensis - T. laxmannii	0	17	79	655	879
T. domingensis - T. shuttleworthii	0	5	81	574	1096
T. latifolia - T. laxmannii	15	19	7	124	1552
T. latifolia - T. shuttleworthii	42	27	1	29	1825
T. laxmannii - T. shuttleworthii	0	17	45	591	893

CHAPTER 4: TIME IN SYMPATRY CORRELATES WITH THE STRENGTH OF REPRODUCTIVE ISOLATION IN HYBRIDISING *TYPHA*

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A version of this chapter is under revision for *Evolution*.

Abstract

In North America, hybridisation between *Typha angustifolia* and *T. latifolia* results in the highly impactful *T.* × *glauca*. In Europe, where its parental taxa are also sympatric, *T.* × *glauca* is scarce, suggesting stronger reproductive isolation on this continent. Using genomic data from the two species in North America and Europe, we reconstructed their demographic histories and characterised barrier loci between them. Demographic modelling suggests that the initial contact between the two species in Europe occurred ~800,000 years ago, indicating sympatry in that region since the Middle Pleistocene. In North America, their contact likely happened within the last centuries and was potentially driven by the human-mediated dispersal of *T. angustifolia*. We identified 47 candidate barrier loci between species in Europe, 6 of which are associated with reproductive functions. No barrier loci were found in North America. Our results suggest that prolonged sympatry can promote the evolution of reproductive barriers, whereas prolonged allopatry can reduce the likelihood of their development. Future studies could help determine whether time in sympatry is a predictor of the strength of reproductive isolation between hybridising taxa. Preventing invasions by hybrid taxa will require limiting the human-mediated dispersal of *Typha* (and other allopatric species) lacking reproductive isolation.

Keywords: barrier loci; demographic histories; evolutionary history; invasion genomics; speciation

Introduction

Hybridisation is an important evolutionary mechanism (Abbott et al. 2013); it can cause the emergence of new species if reproductive barriers develop between parental and hybrid lineages, or lead to genetic swamping and the extinction of parental taxa (Runemark et al., 2019). More commonly, hybridisation results in the formation of hybrid zones—geographic areas where parental and hybrid individuals of one or more generations coexist (Hewitt, 1988). Hybrid zones provide an opportunity to characterise the genetic architecture of interspecific gene flow and reproductive isolation (Taylor & Larson, 2019)

The formation and persistence of hybrid zones are influenced by species boundaries (Larson et al., 2014). Different types of reproductive barriers maintain these boundaries (Harrison & Larson, 2014), with "barrier loci" referring to genetic regions that underlie the traits controlling reproductive isolation between species (Barton & Bengtsson, 1986). Barrier loci can act prezygotically by impeding interbreeding events (Elmer, 2019), or postzygotically by making hybrids sterile, inviable, or unfit (Stankowski & Ravinet, 2021). Prezygotic barrier loci are often under divergent selection (Wu, 2001). For instance, in *Heliconius* butterflies, the optix and WntA wing-patterning loci govern mate recognition and limit interspecific courtship (Merrill et al., 2019). Postzygotic barrier loci frequently arise from intrinsic epistatic incompatibilities, as those explained by the Bateson–Dobzhansky–Muller (BDM) model, in which mismatched allele combinations from different loci decrease hybrid fitness (Bateson, 2009; Dobzhansky, 1936; Muller, 1942); in *Icterus* orioles, a large inversion on the Z chromosome accumulates alleles that cause hybrid breakdown (Walsh et al., 2023).

Understanding the role of barrier loci to reproductive isolation is key to explaining how species boundaries evolve.

Different approaches have been used to investigate the loci that affect reproductive isolation between taxa (e.g., Moyle and Payseur 2009; Schumer et al. 2014; Haenel et al. 2021). In recent years, methods for identifying barrier loci from genome-wide sequencing data have been developed (Burban et al., 2024; Laetsch et al., 2023). These methods use coalescent-based simulations and explicitly incorporate demographic inferences (i.e., divergence times, population sizes, migration rates) (Fraïsse et al., 2021). Demographic-informed approaches to identify barrier loci overcome two limitations of methods based on genomic scans: (i) that the genomic landscapes of diversity and differentiation (e.g., F_{ST}, d_{XY}, π) can emerge from evolutionary forces not directly related to reproductive isolation (e.g., historical bottlenecks, genetic drift, local adaptations), and (ii) that there is no universal summary statistic for barrier loci, as the emergence of these barriers depends on multiple factors, reflected on multiple summary statistics. Identifying barrier loci through demography-aware methods can provide insights into how reproductive isolation evolves throughout species' histories.

Across the Great Lakes, Prairie Pothole, and Midwestern regions of North America, the hybrid cattail *Typha* × *glauca* is a highly impactful invader (reviewed in Bansal et al. 2019). Hybrid cattails outcompete their parental species—*T. angustifolia* and *T. latifolia*—(Freeland et al., 2013, 2024; Geddes et al., 2021; Pieper et al., 2020; Zapfe & Freeland, 2015), reduce biodiversity and alter freshwater functions (Angeloni et al., 2006; Boers et al., 2007; Farrer & Goldberg, 2014; Tuchman et al., 2009), and are expanding across their already broad range (Joyee et al., 2024). Contrastingly, *T.* × *glauca* is scarce in Europe and has not been reported in Asia, where its parental species also co-occur

(Ciotir et al., 2017; Nowińska et al., 2014; Volkova & Bobrov, 2022; Zhou et al., 2016). A key difference in the history of both parental species in North America and Europe is a longer time inhabiting Europe, where they might have been sympatric since the emergence of the younger, *T. latifolia*, ~5.7 Ma (Zhou et al., 2018), or once it recovered from a severe demographic decline and subsequently expanded ~800,000 years ago (Aleman et al., 2025). *Typha latifolia* arrived in North America via the Beringian Land Bridge from Eastern Eurasia—the centre of origin of *Typha*—between the Late Miocene and Early Pliocene, i.e., from 5.7 to 3.5 Ma (Zhou et al., 2018), while *T. angustifolia* likely arrived centuries ago, potentially due to human dispersal (Ciotir et al., 2013). Although we cannot rule out the possibility that the two species might have experienced contact in East Eurasia before *T. latifolia* arrived in North America, the available information indicates that *T. latifolia* has been isolated from *T. angustifolia* since it entered North America and until *T. angustifolia* was introduced (Ciotir et al., 2013; Ciotir & Freeland, 2016).

The broad distribution of the *T.* × *glauca* hybrid swarm in North America suggests that reproductive barriers between *T. latifolia* and *T. angustifolia* are weak on this continent. In contrast, the scarcity of *T.* × *glauca* in Europe suggests that there are strong reproductive barriers between its two parental species in this region. Aleman et al. (2025) identified introgressive hybridisation from *T. latifolia* to *T. angustifolia* in North America and Europe, which, together with the scarcity of *T.* × *glauca* in Europe, suggests that the two species hybridised in the past in Europe and reproductive barriers emerged later.

We hypothesised that (i) in North America, the time in isolation that *T. latifolia* experienced before *T. angustifolia* entered the continent reduced the possibility for natural selection to drive the evolution of reproductive isolation between the two species,

whereas (ii) in Europe, their longer coexistence and historical hybridisation led to the development of reproductive barriers. Therefore, we expected to identify more barrier loci between the two species in Europe than in North America. Characterising these loci could help inform why *T.* × *glauca* is a highly impactful invader in some parts of North America but not in Europe. Understanding what limits hybridisation between the two species in Europe should receive special consideration, since *Typha* is frequently transported between continents via garden centres (Ciotir & Freeland, 2016). If barriers to hybridisation are genetic—rather than ecological—the human-mediated movement of *Typha* could lead to the contact of species lacking reproductive isolation, facilitating future invasions by novel hybrids. From a theoretical standpoint, the time in sympatry for *T. angustifolia* and *T. latifolia* in North America and Europe is uniquely positioned to test whether barriers to hybridisation could be stronger between taxa that have experienced prolonged periods of contact than those whose time in sympatry is very recent.

Materials and Methods

Genomic data processing

Raw sequencing data from *T. angustifolia* (36 from North America, 16 from Europe) and *T. latifolia* (47 from North America, 23 from Europe) were obtained from Aleman et al. (2025) and supplemented with data from additional samples (Figure 4.1; Supplementary Table S4.1), generated following Aleman et al., (2024). The quality of the demultiplexed raw sequences was evaluated using FastQC 0.11.9 (Andrews, 2017) and MultiQC 1.14 (Ewels et al., 2016). Read pairing and adapter trimming were performed using Trimmomatic 0.39 (Bolger et al., 2014), removing reads shorter than 100 bp after trimming. Cleaned reads were mapped to the *T. latifolia* nuclear (GenBank accession JAIOKV000000000.2 (Widanagama et al., 2022)) and chloroplast (GenBank accession

NC_013823 (Guisinger et al., 2010)) genomes using BWA 0.7.17 (Li & Durbin, 2009). Mapping statistics were generated using SAMtools 1.15.1 (Li et al., 2009). Genotyping was conducted using ANGSD 0.93 (Korneliussen et al., 2014). SNPs were called for all samples, requiring minimum mapping and sequencing scores of 20, as well as a minimum *p*-value of 1^{e-6}. Sites mapped to the plastome were removed using VCFtools 0.1.16 (Danecek et al., 2011). Two SNP datasets were generated based on two missing data thresholds across all samples (i.e., without considering taxonomy or geographic location): 20% for the genetic structure analysis, to ensure high-confidence genotypes for accurate sample classification (Yi & Latch, 2022), and 50% for the demographic history and barrier locus characterisation, to maximize SNP retention, since coalescent-based methods explicitly model genotype uncertainty and require larger datasets for robust parameter estimation (Fraïsse et al., 2021). To confirm the samples' species membership, their genetic relationships were evaluated by a neighbour-joining (NJ) tree analysis, conducted using Plink 1.9 (Purcell et al., 2007).

Genetic composition of the T. × glauca hybrid swarm

To assess the genetic composition of hybrids across the *T. × glauca* swarm, sequencing data were generated from 38 hybrid samples following Aleman et al. (2024). Thirty-six of these samples were from North America, whereas only two were found in Europe, despite an extensive collecting trip that aimed to maximise the number of sampled hybrids (Ciotir et al., 2017). In North America, the 36 hybrids were collected during two previous studies (Pieper et al., 2020; Tisshaw et al., 2020) (Figure 4.1; Supplementary Table S4.1). Raw sequences were quality-inspected, cleaned, and mapped to the *T. latifolia* reference genome following the *Genomic data processing* steps above. SNPs were called for the 42,167 sites (see *Results*) used in the genetic structure analysis

for *T. angustifolia* and *T. latifolia*. The nuclear composition of hybrids was assessed using ADMIXTURE 1.3.0 (Alexander & Lange, 2011), which tested *K* clusters from 1 to 10 for all samples in this study. The optimal *K* number was chosen via a cross-validation procedure. To assess whether hybrids had the plastome of *T. angustifolia* and *T. latifolia*, the plastomes of all samples in this study were assembled using the reference-based approach in Aleman et al. (2024), and SplitsTree4 (Huson & Bryant, 2006) was used to reconstruct a phylogenetic network for all samples in this study.

Demographic history and characterisation of barrier loci

To reconstruct the demographic history of *T. angustifolia* and *T. latifolia* in North America and Europe, and to identify barrier loci between the two species, RIDGE (Burban et al., 2024) was run between species within and between continents (e.g., North American *T. angustifolia* with European *T. latifolia*); i.e., four independent analyses were conducted (Table 4.1). No hybrids were included in these analyses. Before the demographic inferences, several summary statistics were computed in 5 kb windows, including the genetic differentiation between species through F_{ST} (Hudson et al., 1992), d_{XY} (Nei & Miller, 1990), d_a (Nei & Li, 1979), and their joint Site Frequency Spectrum jSFS (Wakeley & Hey, 1997), and the diversity within each species through π (Nei & Li, 1979), Watterson's θ (Watterson, 1975), and Tajima's D (Tajima, 1989). Four demographic scenarios of divergence between species were simulated using an Approximate Bayesian Computation framework: strict isolation, ancestral migration, secondary contact, and isolation–migration. These scenarios included both homogeneous and heterogeneous population sizes, as well as interspecific gene flow rates over time (Figure 4.2). The prior distributions of species' divergence times and effective population sizes were determined based on the summary statistics observations (Supplementary

Table S4.2). To avoid having more than two orders of magnitude between the prior bounds of the time at which the two species diverged from a common ancestor, the minimum divergence time was adjusted to two-thirds of the estimated maximum divergence time. However, the priors for the minimum and maximum population sizes were left unadjusted. During each of the four independent analyses, 7.5 M sites were computed over 560 simulations.

Simulation results were used to build a demographic hypermodel, where each parameter was obtained from the weighted likelihood of the four scenarios tested. The hypermodel was used to simulate 1.5 M barrier and non-barrier loci, i.e., with $m = 0$ and $m > 0$, respectively, where m is the effective gene flow rate between species. The probability that a simulated locus was accurately identified as a barrier or non-barrier locus was estimated to obtain a false-positive and false-negative correction (Q). The simulation results were used to calculate the likelihood that a real-life locus was a barrier to hybridisation. The identification of a locus (i.e., a 5 kb window) acting as a barrier to hybridisation was determined by a Bayes Factor of 10, given the correction Q (known as "BF_approxQ"); this meant that a barrier locus was at least 10 times more probable to be a barrier to hybridisation than a non-barrier locus. Barrier loci identified in each run were compared, and summary statistics for barrier and non-barrier loci were evaluated.

BEDTools Intersect (Quinlan & Hall, 2010) was used to identify the genes associated with barrier loci from the *T. latifolia* annotation (Widanagama et al., 2022). The ratio of nonsynonymous to synonymous substitution rates (dN/dS) for all barrier loci was calculated among the four groups of taxa (i.e., the two species from the two continents) using MEGA 12 (Kumar et al., 2024). Barrier loci that could not be associated

with genes from the *T. latifolia* annotation were screened using BLAST+ to search for homologous genes (Camacho et al., 2009).

Results

Using 42,167 SNPs, the NJ tree confirmed that in each continent, parental samples clustered into two groups (Figure 4.1). The ADMIXTURE analysis, including parental and hybrid samples, established the most likely number of genetic clusters as two ($K = 2$). In North America, 19 hybrids were F1s and 17 were backcrosses with T. *angustifolia*, with a nuclear proportion of ~70% from this species; all hybrids (F1s and backcrosses) had the *T. angustifolia* plastome. In Europe, one hybrid was an F1 and had the *T. latifolia* plastome, and the other was a backcross with both a higher nuclear proportion (70%) and the *T. angustifolia* plastome (Figure 4.1).

The demographic history and barrier locus analyses used 6,735,717 SNPs. In each of the four demographic history reconstructions of *T. angustifolia* and *T. latifolia*, secondary contact and isolation–migration received the highest likelihood weights; these scenarios consistently indicated heterogeneous rates of interspecific gene flow and population sizes over time (Figure 4.2, Table 4.1, Supplementary Table S4.3). The four reconstructions successfully fitted the observed data, with a posterior goodness-of-fit value greater than 0.05. The estimates of divergence and contact times were consistent across all analyses. The mean divergence time between the two species was estimated to be ~1.25 M generations ago, and the mean time of secondary contact was ~400,000 generations ago.

A total of 49 barrier loci between *T. angustifolia* and *T. latifolia* were identified, 47 of those in Europe (Figure 4.3). No barrier loci were identified in North America. Two barrier loci were found between North American *T. angustifolia* and European *T. latifolia*

(one of those was also a barrier locus in Europe), and two barrier loci were found between European *T. angustifolia* and North American *T. latifolia* (one of those was also a barrier locus in Europe).

Of the total 49 barrier loci, 12 were annotated to 10 genes (3 were to the same gene) from the *T. latifolia* reference, and BLAST predicted other 34 to be genes (Supplementary Table S4.4). In the 47 barrier loci from Europe, European *T. angustifolia* had a mean diversity ($\pi = 0.0028$) lower than the rest of its genome ($\pi = 0.0073$), and no diversity differences were observed the other three groups of taxa; 10 barrier loci were highly differentiated for F_{ST} between species with values above the 95th percentile, of those, 1 of those was also highly differentiated for d_{XY} and 3 were genes; of the other 7 genes found in barrier loci, 1 was under purifying selection between species in Europe ($dN/dS < 1$), and 1 was under positive selection ($dN/dS > 1$) in all groups of taxa except European *T. angustifolia*.

Discussion

The number and identity of loci associated with species' reproductive isolation can shed light on how species boundaries evolve along their genomes and through time (Gavrilets, 2003; Ravinet et al., 2017). Additionally, understanding the evolutionary histories of the parental species of *T.* × *glauca* and the mechanisms that promote or limit their interbreeding can help us prevent further invasions by novel *Typha* hybrids. This study provides insights into the demographic histories and barriers to hybridisation between *T. angustifolia* and *T. latifolia*; our results suggest that in Europe, where very few hybrids have been identified, secondary contact between the two parental species occurred ~400,000 generations ago. *Typha angustifolia* likely entered North America

within the last centuries, where it interbreeds with *T. latifolia*, and their hybrids are generally invasive.

Hybridisation between *T. angustifolia* and *T. latifolia* in North America is highly asymmetric, with *T. angustifolia* often being the maternal parent (Ball & Freeland, 2013; Pieper et al., 2017). Consistent with this, the 19 North American F1 hybrids in this study had the *T. angustifolia* plastome. Since hybrid classes go beyond F1, the *T.* × *glauca* hybrid zone is a swarm; F1s can backcross bidirectionally with *T. angustifolia*, asymmetrically with *T. latifolia* as the paternal parent, or reproduce with other F1s (Bhargav et al., 2022; Freeland et al., 2013). Pieper et al. (2017) observed that no seeds are set when *T. latifolia* is pollinated by hybrid cattails. In line with this, we identified 17 backcrosses with a higher nuclear proportion and the plastome of *T. angustifolia*; this observation suggests that *T. angustifolia* is almost always present during backcrossing events. Asymmetric hybridisation can reflect early reproductive isolation, as seen in *Rhododendron delavayi* and *R. cyanocarpum* azaleas (Ma et al., 2016), *Primula vulgaris* primroses and *P. veris* cowslips (Keller et al., 2021), or *Erysimum mediohispanicum* and *E. nevadense* wallflowers (Abdelaziz et al., 2021). However, across the North American Great Lakes, Prairie Pothole, and Midwest, hybrids are generally invasive and outnumber their parental taxa (Bansal et al., 2019; Geddes et al., 2021; Tangen et al., 2022)—suggesting that this prospective barrier is ineffective.

In this study, only two hybrids were observed in Europe, despite an extensive collecting trip that aimed to maximise the number of sampled hybrids (Ciotir et al., 2017). One of these hybrids was an F1 and had the *T. latifolia* plastome. Freeland et al., (2013) and Pieper et al., (2017) identified occasional *T. latifolia* cpDNA in hybrids. The presence of the *T. latifolia* plastome in some European hybrids raises the question of whether

another mechanism of reproductive isolation between the two species in Europe could be related to the frequency of hybridisation events involving *T. latifolia* as maternal parent.

While *T. angustifolia* and *T. latifolia* are not sister species, they belong to two sister clades, and *T. latifolia* appears to have originated between 2 and 3 M generations ago (Aleman et al., 2025), i.e., ~4 to 6 Mya, in line with Zhou et al (2018), assuming a 2-year generation time (Yeo, 1964). Our underestimation of their divergence time (~1.25 M generations ago) is partially explained by the fact that they are not sister species; the missing branches between them might have inflated the species coalescence rates, making their divergence appear younger. Another factor contributing to this underestimate is that coalescent rates can anchor to periods of elevated demographic sizes (Wang et al., 2016); consistent with this, our divergence time estimation overlaps with a historical demographic peak observed in *T. latifolia* (Aleman et al., 2025). The successful posteriors goodness-of-fit (>0.05), similitude among the time of divergence and secondary contact estimations across all analyses and in this study (~1.25 M generations ago), and the estimated time of secondary contact between the two species (~400,000 generations ago)—coincident with the most recent demographic expansion experienced by *T. latifolia* (Aleman et al., 2025)—support the validity of our inferences.

Our estimated time of secondary contact between *T. angustifolia* and *T. latifolia* in North America was ~430,000 generations ago, which predates the potential introduction of *T. angustifolia* to North America (Ciotir et al., 2013). When *T. angustifolia* entered North America, it would have carried the genomic signatures of contact with *T. latifolia* in Europe, which is how this date should be interpreted. It is expected that a coalescence approach would identify this historical contact in Europe, which reflects historical gene

flow into *T. angustifolia* and is in line with Aleman et al. (2025), who observed introgressive hybridisation from *T. latifolia* into *T. angustifolia* in Europe.

Typha latifolia underwent a cycle of demographic declines and expansions between ~2 M and 400,000 generations ago (equivalent to ~4 M and 800,000 years) in North America and Europe (Aleman et al., 2025). Consistent with this, we estimated that *T. angustifolia* and *T. latifolia* came into contact ~400,000 generations ago (equivalent to ~800,000 years ago), suggesting the two species have been sympatric in Europe since at least the Middle Pleistocene, after the last expansion of *T. latifolia*. After coming into secondary contact, they likely hybridised and later developed reproductive barriers (Aleman et al., 2025).

A total of 47 candidate barrier loci between *T. angustifolia* and *T. latifolia* were identified in Europe, whereas none were identified in North America. This finding supports the hypothesis that reproductive barriers between the two species are stronger in Europe than in North America. A possible explanation for the rise of reproductive barriers between *T. angustifolia* and *T. latifolia* in Europe could be selection against hybrids (Hopkins, 2013; Pfennig, 2016)—future evidence will be necessary to support this idea. Identifying barrier loci in Europe but not in North America also supports the hypothesis that barriers to hybridisation could be stronger between species that have experienced prolonged periods in sympatry than those whose time in sympatry is short; further research could help determine if this is a general pattern of hybridisation. In flycatchers (Ficedula *albicollis* × *F. hypoleuca*) and oaks (*Quercus mongolica* × *Q. liaotungensis*), hybridisation rates are lower in older than younger areas of sympatry (Haavie et al., 2004; Liao et al., 2019). Similar patterns of reduced hybridisation in regions of prolonged sympatry have been observed in *Bombus* bumblebees and *Heliconius* butterflies, where

selection strengthened reproductive barriers over time in contact (Christmas et al., 2021; Lewis et al., 2020).

Of the 47 barrier loci identified in Europe, 12 belonged to 10 genes associated with ubiquitin-mediated regulation and degradation (At4g11680) (Stone et al., 2005), protein modification and assembly (CASP1, CCB2, UGT80A2) (DeBolt et al., 2009; Lyska et al., 2007; Roppolo et al., 2011), membrane traffic and localisation (VAMP714, ALMT12) (Gu et al., 2021; Sanderfoot, 2007; Sasaki et al., 2010), and important regulatory roles (IAA19, CINV1, PAIR2, and PDCD2). Notably, IAA19—which spanned three barrier loci (15 kb) and is under purifying selection between species in Europe—is involved in mediating auxin's effects on plant growth (Kohno et al., 2012); CINV1 is involved in primary root elongation, lateral root formation, floral transition, and pollen development (Barratt et al., 2009); PAIR2 is essential for meiotic chromosome pairing (Nonomura et al., 2006); and PDCD2 is involved in apoptosis (Reape & McCabe, 2008). These genes are good candidates for causing reproductive barriers, supporting the role of barrier loci in contributing to reproductive isolation in Europe by impeding interbreeding events or making *T.* × *glauca* inviable or unfit.

The absence of barrier loci between *T. angustifolia* and *T. latifolia* in North America is consistent with the broad ranges of *T.* × *glauca* hybrids across the Great Lakes, Prairie Pothole, and Midwestern regions (reviewed in Bansal et al. 2019). On this continent, *T. latifolia* was potentially isolated since its arrival, between 5.7 and 3.5 Ma (Zhou et al., 2018), and until the potential arrival of *T. angustifolia* (Ciotir et al., 2013; Ciotir & Freeland, 2016). This time in isolation appears to have reduced the likelihood of reproductive barriers evolving between the two species, in contrast to Europe, where they have been sympatric for a prolonged time and reproductive barriers emerged after they

hybridised in the past (Aleman et al., 2025). Alternatively, if barriers to hybridisation between the two species arose during a potential contact in Eurasia before *T. latifolia* arrived in North America, these barriers might have been lost during the time *T. latifolia* was isolated. In allopatry, selective pressures against (potentially maladaptive) hybrids are eliminated; without these pressures, local genetic differences driven by drift and adaptations might erode reproductive barriers that once prevented hybridisation (Coughlan & Matute, 2020). This dynamic—where reproductive isolation strengthens in sympatry due to selection against hybrids but weakens in allopatry due to its absence—is a common feature of species with cycles of isolation and contact (Kulmuni et al., 2020).

Conclusions

Contributing to the results from Ciotir et al. (2013), Zhou et al. (2018), and Aleman et al. (2025), we propose that after diverging in allopatry due to drift-driven events ~4 to 6 Mya, *T. angustifolia* and *T. latifolia* experienced secondary contact in Europe ~400,000 generations ago (equivalent to ~800,000 years ago), hybridised, and later developed reproductive isolation (reflected in 47 barrier loci). *Typha latifolia* arrived in North America from East Eurasia between 5.7 and 3.5 Ma and remained isolated until *T. angustifolia* arrived on this continent, potentially due to human dispersal. No barrier loci have arisen between the two species in North America. Future research could apply similar methods to *T. angustifolia* and *T. latifolia* in Eastern Asia—where they do not hybridise, and we predict more barrier loci should be found. Additionally, future research could investigate if selection against hybrids is the cause of reproductive barriers arising between *T. angustifolia* and *T. latifolia* in Europe. The lack of reproductive isolation between these two species in North America suggests that preventing future hybrid

invasions will require limiting the movement of *Typha* and other allopatric species, which most likely lack reproductive barriers.

Acknowledgements

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Figures and Tables

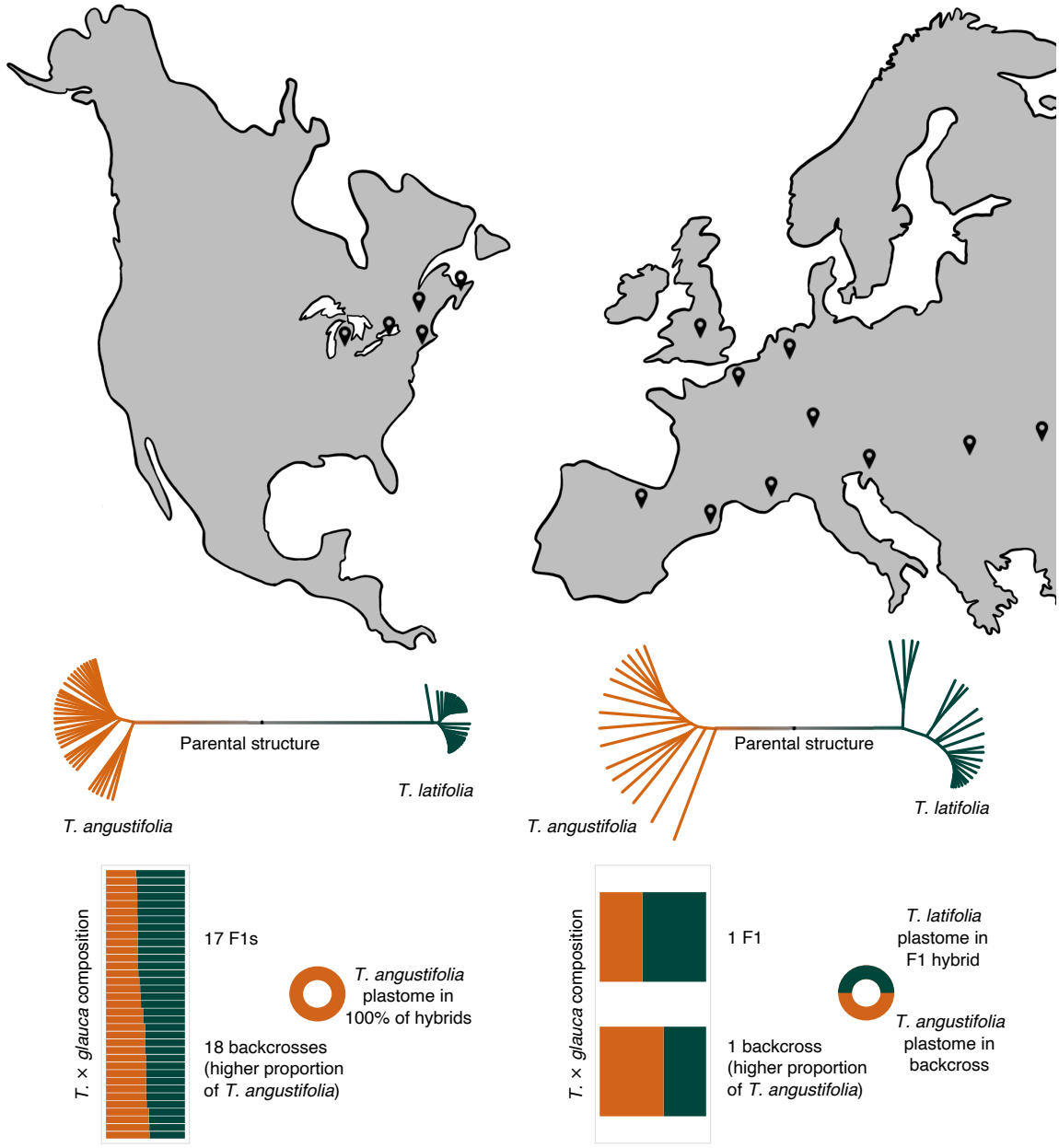

Figure 4.1. Top: Approximate sampling sites (black marks) in this study; sample sizes are not represented graphically. Middle: NJ trees for *Typha angustifolia* and *T. latifolia* in this study; branches represent samples, and colours indicate species, as labelled. Bottom: Genetic composition of *T.* × *glauca* samples across the hybrid zone.

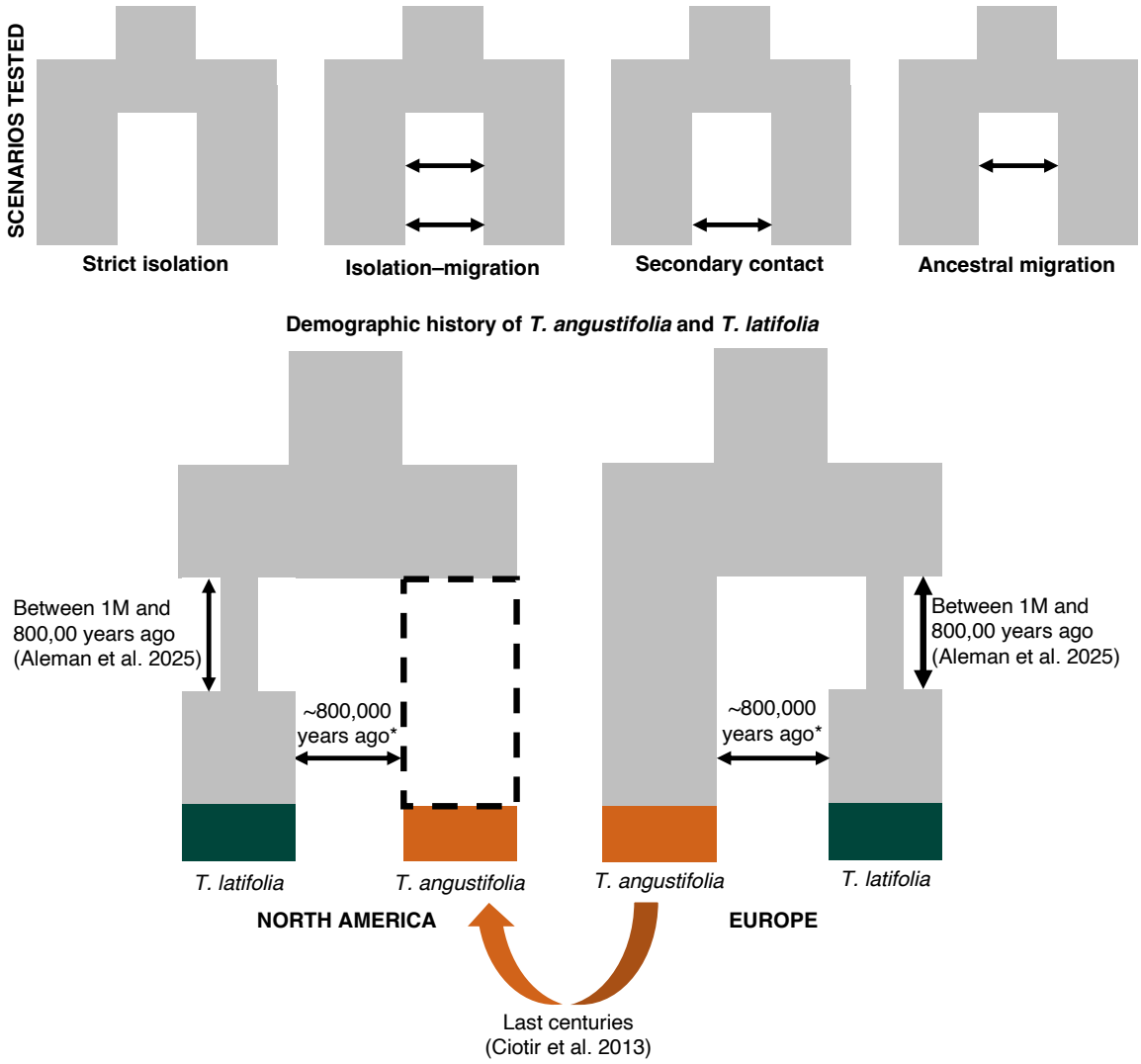

Figure 4.2. Demographic history of *T. angustifolia* and *T. latifolia* in North America and Europe proposed in this study. *Parameter estimated by RIDGE in this study.

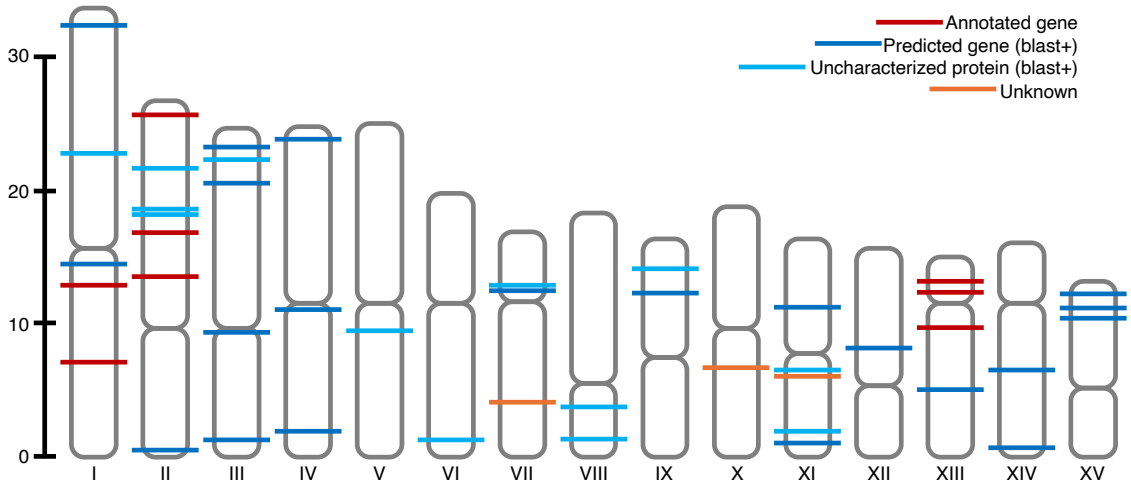

Figure 4.3. Distribution of loci predicted to be responsible for reproductive isolation between *T. angustifolia* and *T. latifolia* in Europe in this study. Centromeres were predicted using RepeatObserver (Elphinstone et al., 2025) with the default settings.

Table 4.1. Demographic history of *T. angustifolia* and *T. latifolia* in North America and Europe. Parameters estimated by RIDGE. *Ciotir et al. (2013) showed that *T. angustifolia* was likely introduced in the last centuries, which is the recognised time of secondary contact between the two species.

	North America	**Europe**	***T. angustifolia* (North America) – *T. latifolia* (Europe)**	***T. angustifolia* (Europe) – *T. latifolia* (North America)**
Demographic scenario with highest likelihood	Isolation–Migration	Secondary Contact	Secondary Contact	Isolation–Migration
Time of divergence (generations)	1,296,184	1,177,131	1,282,128	1,262,273
Time of secondary contact (generations)	430,621*	368,064	379,596	387,936
Number of barrier loci	0	47	0	0

CHAPTER 5: GENERAL DISCUSSION

This thesis integrates genomic and demographic analyses to reconstruct the evolutionary histories of *Typha angustifolia* and *T. latifolia,* providing insights into their hybrid, *T.* × *glauca*, a highly impactful invader across the Laurentian Great Lakes, Prairie Pothole, and Midwestern regions of North America. Our findings reveal that these taxa originated through a series of Late Miocene–Early Pliocene, drift-driven speciation events in allopatry, followed by secondary contact and hybridisation. We also demonstrate that the contemporary dynamics of their hybrid zones appear to be influenced by their time in sympatry, which may have driven the evolution of reproductive isolation in Europe—and possibly in Asia. This work contributes to the relationship between speciation and hybridisation, and provides insights to prevent further invasions by *T.* × *glauca* and other *Typha* hybrids.

The results of Chapter 3 suggest that *T. angustifolia* and *T. latifolia*, and their sister species *T. domingensis* and *T. shuttleworthii,* diverged in allopatry via drift-driven events (rather than by selection-driven ecological divergence with gene flow), likely from *T. laxmannii* or a common ancestor with this species, between ~5 and ~2.5 million generations ago, during the Late Miocene–Early Pliocene. Assuming a 2-year generation time (Yeo, 1964), these events align chronologically with the divergence times reported by (Zhou et al., 2018), which, together with sharp declines in the past effective sizes (N_e) in *T. laxmannii*, are consistent with the predictions of drift-driven speciation (reviewed by Bock et al., 2023). Given that Asia was the centre of origin of *Typha* (Zhou et al., 2018), the Miocene aridification and the Quaternary glacial cycles (Butiseacă et al., 2021; Herbert et al., 2016; Hewitt, 2000; Miao et al., 2012) are potential causes for species divergence. Increased aridity, particularly in mid-latitude regions (Shen et al., 2018),

could have temporarily eradicated wetlands from large areas, leading to bottlenecks in *Typha*. These geoclimatic events have driven divergence in other taxa; for example, in Eastern Asia, intensified aridifications from the Mid-Miocene onwards drove the diversification of mesobuthid scorpions (Shi et al., 2013). In the Western Mediterranean, *Harpactocrates* spiders underwent major diversification events triggered by the Miocene climatic transition, which were further intensified during the Quaternary glacial cycles (Bidegaray-Batista et al., 2014). The *Typha* spp. genomic analyses in this study add to the evidence that speciation events were widespread during the late Miocene and Quaternary periods (e.g., Hewitt, 2000; Kadereit & Abbott, 2021; Steinthorsdottir et al., 2021).

The genetic distance among the *Typha* spp. in this dissertation is short (d_a = ~0.01), i.e., their reproductive isolation is incomplete. Most genetic divergence among these species has arisen neutrally (Chapter 3); however, even under neutral evolution, such a small distance was unexpected. There are at least three possible causes for the short genetic distance among these species: 1. A slow mutation rate (μ), accumulating changes more slowly than other lineages; large census and effective sizes with low genetic diversity—as those observed in Chapter 3—have been associated with slow mutation rates in *Spirodela polyrhiza* duckweeds (Xu et al., 2019) and algae of the genera *Ectocarpus* and *Scytosiphon* (Krasovec et al., 2023). 2. Most genetic variation in *Typha* could have been lost during the bottlenecks that triggered species divergence; severe drift events can cause species to start from a small, genetically homogeneous subset of diversity (Barton & Charlesworth, 1984). 3. Recent or ongoing gene flow, which is consistent with the observation that some species hybridise (Ciotir et al., 2017; Mavrodiev & Kapitonova, 2015; Smith, 1967; Volkova & Bobrov, 2022). The

combination of these causes likely results in a small number of genetic differences accumulating over the millions of years since their split.

Another unexpected observation in Chapter 3 was the minimal role of divergent selection and background selection in driving *Typha* spp. divergence. Most regions where selection was detected among the species in our study were characterised as valleys of balancing selection; this type of selection maintains high levels of polymorphism and low differentiation among species (Guerrero & Hahn, 2017). Genes experiencing balancing selection have been shown to underlie a wide range of phenotypes (Isildak et al., 2021; Promy et al., 2023) and promote adaptation to divergent habitats (Delph & Kelly, 2014; Wu et al., 2017). Numerous studies have identified loci potentially experiencing balancing selection in *Arabidopsis* cress, *Arbutus* madrones, *Populus* poplars, and *Quercus* oaks (Le Veve et al., 2023; Meireles et al., 2017; Rendón-Anaya et al., 2019; Santiso et al., 2016; Shang et al., 2023; Wang et al., 2020). However, background selection is considered the leading type of selection shaping species' genetic variation (Comeron, 2017). Alternatively, other evolutionary processes may be mimicking these patterns. Soni & Jensen (2024) warn how introgressive hybridisation, incomplete lineage sorting, and demographic expansions can lead to an excess of intermediate frequency alleles. Hence, further research will be necessary to determine the prevalence of balancing selection across *Typha*.

The speciation of *T. angustifolia*, *T. domingensis*, and *T. latifolia* followed a scenario of secondary contact with gene flow. After diverging, demographic and range expansions brought species into sympatry, creating at least one widespread hybrid zone (*T.* × *glauca*); although the three species are capable of hybridising (Smith, 1967). Allopatric divergence and hybridisation upon secondary contact have shaped the

evolution of multiple taxa; some plant species following a similar history include the two most widely distributed European species of oaks, *Quercus robur* and *Q. petraea* (Le Provost et al., 2022), the izotes *Yucca capensis* and *Y. valida* (Arteaga et al., 2020) in the Baja California Peninsula, and the sedges *Carex furva* and *C. lucennoiberica* in the Iberian Peninsula (Maguilla et al., 2017), of which geographic isolation and secondary contact were associated with glacial cycles.

The time *T. angustifolia* and *T. latifolia* have been sympatric on different continents appears to be correlated with the strength of reproductive isolation between them (Chapter 4). In North America, where *T. latifolia* may have been isolated since it entered the continent (between 5.7 and 3.5 million years ago) and until the potentially human-mediated arrival of *T. angustifolia* several centuries ago (Ciotir et al., 2017), their hybrid, *T.* × *glauca*, is widely distributed and generally invasive. In Europe, where the two species potentially experienced secondary contact ~800,000 years ago, hybrids are scarce, and genetic barriers to hybridisation have arisen, likely due to selection against hybrids during their prolonged time in sympatry. In Asia—the centre of origin of *Typha*—where the two species have potentially been sympatric for a longer time than in Europe—they do not hybridise, suggesting even stronger reproductive isolation. Future research will be necessary to confirm whether there are more genetic barriers to hybridisation between *T. angustifolia* and *T. latifolia* in Asia than in Europe. A rise of reproductive isolation in sympatry has been observed in *Bombus* bumblebees (Christmas et al., 2021), *Howea* palms (Papadopulos et al., 2019), and *Silene* catchflies (Liu et al., 2020), for which selection strengthened reproductive isolation over time. These results bring up a key question regarding the evolution of reproductive isolation: Are barriers to hybridisation stronger between lineages that diverged under gene flow due to ecological

speciation and/or had long periods of sympatry than those that diverged in allopatry and/or had short or absent periods of sympatry?

Considering that barriers to hybridisation between *T. angustifolia* and *T. latifolia* are genetic, we reiterate the need to limit the human-mediated movement of *Typha*. Secondary contact between historically allopatric taxa has been responsible for hybridisation between lineages with no reproductive barriers, in some cases leading to invasive hybrids (Ellstrand & Schierenbeck, 2000; Mesgaran et al., 2016; Schierenbeck & Ellstrand, 2009; Vilà et al., 2000). The invasions by *T.* × *glauca* in North America—and by hybrids of other recently sympatric species, including *Centaurea stoebe* × *diffusa* knapweeds (Blair & Hufbauer, 2010), *Myriophyllum spicatum* × *sibiricum* watermilfoils (Glisson & Larkin, 2021), and *Tamarix chinensis* × *ramosissima* tamarisks (Gaskin & Kazmer, 2009)—illustrate the risks associated with the human-mediated dispersal of species.

Can species' divergence histories correlate with their strength of reproductive isolation?

Speciation occurs when genetic divergence, the accumulation of genetic differences between lineages, leads to the development of barriers that prevent individuals of different lineages from interbreeding (Coyne & Orr, 2004; Edwards et al., 2020). Genetic divergence can occur under two non-mutually exclusive contexts: allopatric divergence, which involves physical isolation, and ecological divergence, which occurs under gene flow in total or partial sympatry (Sobel, 2016; Sobel et al., 2010). One key question is whether the context in which two species diverge can predict the strength of reproductive isolation between them upon secondary contact (Bock et al., 2023; Briggs & Walters, 2016; Stukenbrock, 2014). Based on the findings of this thesis, we hypothesise

that reproductive isolation may arise more rapidly and be stronger after prolonged periods of divergence in sympatry than after periods of divergence in allopatry. While this idea is not entirely new, evolutionary biologists now have access to genome-wide resources and novel methods to test this hypothesis (Burban et al., 2024; Laetsch et al., 2023), providing a new lens through which to evaluate the genomic landscapes of reproductive isolation.

During allopatric divergence, differences accumulate over time across the genome as a result of changes in the allele frequencies of each independent lineage (Hernández-Hernández et al., 2021). In this case, genetic differentiation is predominantly driven by genetic drift (Coathup et al., 2019; Scheiner & Mindell, 2020). This type of divergence primarily leads to the development of postzygotic barriers (Coyne & Orr, 1989, 1997) due to the accumulation of intrinsic, epistatic incompatibilities (e.g., Bateson–Dobzhansky–Muller). These types of barriers reduce hybrid viability or fertility upon interbreeding (Bateson, 2009; Dobzhansky, 1936; Muller, 1942)

Ecological divergence is primarily driven by divergent selection acting on specific traits and the loci underlying those traits, which leads to assortative mating (Nosil, 2012; Pinho & Hey, 2010). In this context, the accumulation of genetic divergence and reproductive isolation depends on the strength of divergent selection and gene flow (Westram et al., 2022). Species that diverge under gene flow mostly exhibit prezygotic barriers (those that prevent mating or fertilisation) as a result of selection against intermediate, sometimes maladaptive, phenotypes (Sciences et al., 2009).

Baack et al. (2015), Christie et al. (2022), and Lowry et al. (2008) reviews support that prezygotic barriers are stronger than postzygotic barriers. Given that allopatric divergence is mainly associated with postzygotic isolation, and ecological divergence with prezygotic isolation, it can be hypothesised that reproductive isolation may be

stronger after long periods of divergence with gene flow than after divergence in allopatry. This hypothesis also suggests that when species that diverged in allopatry (or experienced long periods of physical isolation after sympatric divergence) are brought into contact, they may be more likely to hybridise. The idea that hybridisation could be more likely to occur when allopatric species experience contact should receive special consideration, as hybridisation can facilitate biological invasions (Ellstrand & Schierenbeck, 2000; Mesgaran et al., 2016; Schierenbeck & Ellstrand, 2009), and because the human-mediated dispersal of species facilitates secondary contact between previously allopatric taxa, potentially promoting hybridisation (Preston & Pearman, 2015; Vallejo-Marín & Hiscock, 2016; Vilà et al., 2000).

Evidence suggests that in some cases, divergence with gene flow can lead to the development of strong prezygotic barriers. Sympatrically diverged *Heliconius melpomene* and *H. cydno* butterflies exhibit distinct wing patterns and other ecological differences that promote assortative mating (Merrill et al., 2010); their hybrids are rare in the wild (Mallet et al., 2007), and hybrid females are sterile (Naisbit et al., 2002). On Lord Howe Island, the palms *Howea belmoreana* and *H. forsteriana* diverged under continuous gene flow (Osborne et al., 2019); Papadopulos et al. (2019) identified 47 genomic regions underlying their divergence, and several of these regions harbour adaptive genes related to key ecological traits (e.g., salt and drought tolerance) and contribute to strong reproductive isolation. Jokinen et al. (2019) observed that two flounder species (*Platichthys flesus* and *P. solemdali*) in the Baltic Sea split under continuous gene flow. One species spawns buoyant pelagic eggs in deep, saline basins, while the other lays demersal eggs in shallower waters (Momigliano et al., 2018); the evolution of these differences has driven a strong rise in their reproductive isolation. Contrastingly, some

studies have observed divergence without gene flow in hybridising species. In a geographically widespread *Crotalus* rattlesnake clade, Schield et al. (2019) observed that species divergence has been primarily shaped by drift in geographic isolation, followed by secondary contact and hybridisation. White-tailed deer (*Odocoileus virginianus*) and mule deer (*O. hemionus*) diverged without gene flow during the Pleistocene; genome-wide analyses revealed signatures of allopatric divergence (Kessler et al., 2023).

In *Quercus* oaks (Liao et al., 2019) and *Typha* cattails (Chapter 4), reproductive isolation between ancient and recent contact zones exemplifies how time in sympatry can correlate with the strength of reproductive isolation between species. *Quercus mongolica and Q. liaotungensis* diverged in allopatry (Chen et al., 2021); reproductive barriers are absent in a recent contact zone, and strong in an older contact zone (Liao et al., 2019). In Chapter 4, we demonstrated how time in sympatry correlates with the strength of reproductive isolation in *Typha angustifolia* and *T. latifolia* in Europe and North America. These studies suggest that in some cases, prolonged sympatry can promote the evolution of reproductive barriers, whereas prolonged allopatry can reduce their likelihood.

Concluding remarks

This thesis demonstrates how the divergence and demographic histories of species influence their potential for hybridisation. Drift-driven speciation events in multiple *Typha* spp., likely driven by Miocene aridification and Quaternary glacial cycles, resulted in the formation of lineages with incomplete reproductive isolation; this exemplified how divergence in prolonged allopatry can enhance hybridisation. Characterising barrier loci between *T. angustifolia* and *T. latifolia* in two areas of sympatry showed how long periods in sympatry (~800,000 years) can lead to the evolution of reproductive isolation. Future studies could integrate phenotypic and genomic data to investigate the barrier loci

identified in this thesis. For instance, controlled fertilisation and germination experiments in *T. angustifolia* and *T. latifolia*, as well as characterising barrier loci between the two species in Asia, where no hybrids have been found, could complement our findings of stronger genetic reproductive isolation in regions of longer sympatry. Conservation efforts informed by population genetics could help us identify lineages vulnerable to interbreeding upon secondary contact, allowing us to prevent invasions by novel hybrids.

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APPENDIX

Supplementary materials for Chapter 2

Supplementary Table S2.1. Sampling identifiers, geographic coordinates, species membership, and genetic admixture status of the 140 *Typha* collected for this study.

Sampling ID	Latitude (approximate)	Longitude (approximate)	Species	Genetic admixture
BHP1	50.79	-119.21	*T. latifolia*	No
BJL2	50.57	-119.61	*T. latifolia*	No
BJL3_1	50.57	-119.61	*T. latifolia*	No
BRT2B	44.74	-63.24	*T. angustifolia*	No
BUN1	53.89	-122.81	*T. latifolia*	No
C05TL	52.72	-1.37	*T. latifolia*	No
CA13_TL	44.85	7.71	*T. latifolia*	No
CA3TL	47.68	22.46	*T. latifolia*	No
CC1_TL	50.77	2.31	*T. latifolia*	No
CL10TA	50.77	2.31	*T. angustifolia*	Yes
COTL6	52.72	-1.37	*T. latifolia*	No
CY6ATA	51.43	-0.11	*T. angustifolia*	No
CY7ATA	51.43	-0.11	*T. angustifolia*	Yes
CYTZ_4ATD	-6.79	39.21	*T. domingensis*	Yes
E74B	45.03	-63.50	*T. angustifolia*	Yes
EDD_44_1	47.37	-68.33	*T. latifolia*	No
EDW_2_15	47.37	-68.33	*T. latifolia*	No
EDW_3_6	47.37	-68.33	*T. latifolia*	No
EL14_TA	52.44	5.85	*T. latifolia*	No
ELTA_01	52.44	5.85	*T. angustifolia*	No
ELTA_08	52.44	5.85	*T. angustifolia*	No
FRD47_2	45.96	-66.64	*T. latifolia*	No
FRTD_1	41.64	13.34	*T. domingensis*	Yes
GORE_3	43.68	-70.44	*T. latifolia*	No
HAW_03_12	44.60	-63.55	*T. latifolia*	No
HAW_10_9	44.60	-63.55	*T. latifolia*	No
HM3TL	47.51	19.04	*T. latifolia*	No
HOR_6	48.14	26.51	*T. latifolia*	No
HUF_13TA	47.69	17.65	*T. angustifolia*	No
HUS_TL1	47.51	19.04	*T. latifolia*	No
IP2	44.46	-64.32	*T. latifolia*	No
IR_36	44.97	-64.06	*T. latifolia*	No
IR_39	44.97	-64.06	*T. latifolia*	No
IR_41	44.97	-64.06	*T. latifolia*	No
KL14_TL	46.64	14.31	*T. latifolia*	No
KL1TL	46.64	14.31	*T. latifolia*	No
LIETD_01	42.21	2.61	*T. domingensis*	Yes
LIETD_12	42.21	2.61	*T. domingensis*	Yes
LiETL_11	42.21	2.61	*T. latifolia*	No
M23	45.08	-64.49	*T. latifolia*	No
M30	45.08	-64.49	*T. latifolia*	No

M37	45.08	-64.49	*T. latifolia*	No
M85	45.08	-64.49	*T. latifolia*	No
MBNK_2_8	51.51	-97.00	*T. latifolia*	No
MOD_01_0	46.98	-70.55	*T. latifolia*	No
MOD_02_1	46.98	-70.55	*T. latifolia*	No
MOD_04_1	46.98	-70.55	*T. latifolia*	No
MOD_05_2	46.98	-70.55	*T. latifolia*	No
MOD_07_0	46.98	-70.55	*T. angustifolia*	No
MOD_08_1	46.98	-70.55	*T. latifolia*	No
MOD_14_0	46.98	-70.55	*T. latifolia*	No
MOD_16_1	46.98	-70.55	*T. latifolia*	No
MOD_17_1	46.98	-70.55	*T. latifolia*	No
MOD_18_0	46.98	-70.55	*T. latifolia*	No
MOD_25_2	46.98	-70.55	*T. latifolia*	No
MOD_26_2	46.98	-70.55	*T. latifolia*	No
MOD_29_1	46.98	-70.55	*T. latifolia*	No
MOD_35_3	46.98	-70.55	*T. latifolia*	No
MOD_45_2	46.98	-70.55	*T. latifolia*	No
MOD_54_0	46.98	-70.55	*T. latifolia*	No
NBU_6	42.53	2.83	*T. domingensis*	Yes
OR_TA4	43.28	-2.13	*T. domingensis*	Yes
OR_TL5	43.28	-2.13	*T. latifolia*	No
P6	45.41	-64.33	*T. latifolia*	No
P9	45.41	-64.33	*T. latifolia*	No
PA2	33.19	-112.15	*T. domingensis*	No
PIW_04_6	43.84	-79.10	*T. angustifolia*	No
PIW_09_1	43.84	-79.10	*T. angustifolia*	No
PIW_13_0	43.84	-79.10	*T. angustifolia*	No
PIW_16_2	43.84	-79.10	*T. angustifolia*	No
PIW_24_3	43.84	-79.10	*T. angustifolia*	Yes
PIW_25_3	43.84	-79.10	*T. angustifolia*	Yes
PWE19TL	51.62	-3.94	*T. latifolia*	No
RGT2	45.48	-74.30	*T. angustifolia*	No
SAD_17_3	45.27	-66.09	*T. latifolia*	No
SP2_TA	52.41	4.68	*T. angustifolia*	No
TAKT_1	45.27	37.38	*T. angustifolia*	No
TAKT_2	45.27	37.38	*T. angustifolia*	Yes
TALD_1	55.73	37.61	*T. angustifolia*	Yes
TAVG_1	55.32	40.37	*T. angustifolia*	Yes
TAVG_2	55.32	40.37	*T. angustifolia*	Yes
TA_01_F	26.56	-81.95	*T. latifolia*	No
TB7	44.39	-64.25	*T. latifolia*	No
TDD_04	43.09	47.46	*T. domingensis*	Yes
TDD_08	43.09	47.46	*T. domingensis*	Yes
TDD_09	43.09	47.46	*T. domingensis*	Yes
TDD_15	43.09	47.46	*T. domingensis*	Yes
TDD_16	43.09	47.46	*T. latifolia*	No

TDD_17	43.09	47.46	*T. latifolia*	No
TDD_18	43.09	47.46	*T. domingensis*	No
TDD_22	43.09	47.46	*T. domingensis*	No
TDD_30	43.09	47.46	*T. domingensis*	No
TDD_32	43.09	47.46	*T. domingensis*	Yes
TDD_33	43.09	47.46	*T. domingensis*	Yes
TDD_35	43.09	47.46	*T. latifolia*	No
TDD_36	43.09	47.46	*T. latifolia*	Yes
TDD_37	43.09	47.46	*T. latifolia*	No
TDD_39	43.09	47.46	*T. latifolia*	No
TDD_40	43.09	47.46	*T. domingensis*	No
TDD_41	43.09	47.46	*T. latifolia*	No
TDD_42	43.09	47.46	*T. latifolia*	No
TDD_43	43.09	47.46	*T. angustifolia*	Yes
TDD_44	43.09	47.46	*T. domingensis*	Yes
TDD_46	43.09	47.46	*T. domingensis*	No
TDD_47	43.09	47.46	*T. domingensis*	No
TDD_49	43.09	47.46	*T. angustifolia*	Yes
TDD_50	43.09	47.46	*T. domingensis*	No
TDD_51	43.09	47.46	*T. domingensis*	Yes
TDD_52	43.09	47.46	*T. domingensis*	Yes
TDD_53	43.09	47.46	*T. domingensis*	No
TDD_54	43.09	47.46	*T. domingensis*	Yes
TLIK_01	45.09	147.89	*T. latifolia*	No
TLIK_03	45.09	147.89	*T. latifolia*	No
TLIK_06	45.09	147.89	*T. latifolia*	No
TLIK_15	45.09	147.89	*T. latifolia*	No
TLIK_24	45.09	147.89	*T. latifolia*	No
TLMK_1	45.21	38.89	*T. latifolia*	No
TLPK_1	45.21	38.89	*T. latifolia*	No
TLSK_02	47.06	142.53	*T. latifolia*	No
TLSK_04	47.06	142.53	*T. latifolia*	No
TLSK_11	47.06	142.53	*T. latifolia*	Yes
TLSK_12	47.06	142.53	*T. latifolia*	No
TMO2_7	46.09	-64.78	*T. latifolia*	No
TXKT_1	45.29	37.41	*T. angustifolia*	Yes
TXKT_2	45.29	37.41	*T. angustifolia*	Yes
TXKT_3	45.29	37.41	*T. angustifolia*	Yes
TXKT_4	45.29	37.41	*T. angustifolia*	Yes
TXKT_6	45.29	37.41	*T. angustifolia*	Yes
TXVG_1	55.32	40.37	*T. angustifolia*	Yes
VFW6	49.63	-125.40	*T. latifolia*	No
Vikram_25	44.30	-78.32	*T. angustifolia*	Yes
Vikram_33	44.30	-78.32	*T. angustifolia*	No
Vikram_76	44.30	-78.32	*T. latifolia*	No
Vikram_94	44.30	-78.32	*T. angustifolia*	Yes
WBD20_1	44.28	-84.23	*T. angustifolia*	No

WBD_27_4	44.28	-84.23	*T. angustifolia*	No
WBD_28_3	44.28	-84.23	*T. angustifolia*	No
WBD_32_1	44.28	-84.23	*T. angustifolia*	Yes
WBD_33_1	44.28	-84.23	*T. angustifolia*	No
WBW5_4	44.28	-84.23	*T. latifolia*	No

Supplementary materials for Chapter 3

Supplementary information – Methods

DNA Extraction

Leaf tissue was dried in desiccant silica beads and stored at -20 °C. Dried leaf material was ground with a Retsch® MM300 mixer mill (Haan, Germany). We used 25–30 mg of semi-fine powder from each sample with the EZNA Plant DNA kit (Omega-BioTek) or the Fastpure Plant DNA Isolation Mini Kit (Nanjing Vazyme Biotech, China) protocols for dried material, with a final elution volume of 100 µL.

Nextera XT libraries

DNA was quantified using a Qubit fluorometer (Thermo Fisher Scientific) and calculated as the mean of three independent readings for each sample. All samples were either standardised to 2 ng/µL by dilution with nuclease-free water or left undiluted if their concentrations were less than 2 ng/µL (0.4–1.9 ng/µL). Each sample was tagmented with the Illumina Tagment DNA enzyme (TD) and buffer kit (small kit, #20034210). The reagent volumes for the library preparation were 5.5 µL of 5× TD buffer, 0.5 µL of 1× TD enzyme, and 4 µL of DNA (standardised or undiluted), with all reagents kept on ice during preparation. Samples were incubated at 55 °C for 10 minutes and then allowed to cool to room temperature for 5 minutes. Subsequently, 5 µL of each sample was run on an agarose gel to confirm the efficacy of the tagmentation reaction, as evidenced by the presence of visible smears. The tagmented DNA was then amplified using unique dual indexing based on combinations from a total of 24 N7 (47 bases) and 8 S5 (51 bases) adapters (Alpha DNA, Canada). The PCR cocktail included 0.2 µM of each index, 0.5 U of KAPA HiFi HotStart DNA polymerase (Roche), 12.5 µL of 5× KAPA reagent, 5 µL of tagmented DNA, and 6.5 µL of nuclease-free water to a final volume of 25 µL. The PCR cycle consisted of 72 °C (3 minutes), 95 °C (30 seconds), and 14 cycles of 95 °C (10 seconds), 55 °C (30 seconds), and 72 °C (30 seconds). Again, visible smears confirmed amplification success after running 5 µL of the PCR product on an agarose gel; then, 10 µL of each sample was pooled, and the remaining PCR products were stored at -20 °C. The pooled library was purified using a QIAquick PCR purification kit (QIAGEN) according to the manufacturer's protocol, with a final elution in 50 µL of elution buffer. The library was quantified using a D1000 Tapestation assay (Agilent Technologies, USA) and a Qubit fluorometer (Thermo Fisher Scientific).

Supplementary Table S3.1. Sampling identifiers, geographic coordinates, and species memberships of the samples collected in this study.

Sampling ID	Latitude (approximate)	Longitude (approximate)	Species
BHP1	50.79	-119.21	*T. latifolia*
BJL2	50.57	-119.61	*T. latifolia*
BJL3_1	50.57	-119.61	*T. latifolia*
BRT2B	44.74	-63.24	*T. angustifolia*
BUN1	53.89	-122.81	*T. latifolia*
C05TL	52.72	-1.37	*T. latifolia*
CA13_TL	44.85	7.71	*T. latifolia*
CA3TL	47.68	22.46	*T. latifolia*
CC1_TL	50.77	2.31	*T. latifolia*
CC2_TL	50.77	2.31	*T. latifolia*
CIUU13TA	43.76	24.93	*T. angustifolia*
CL10TA	50.77	2.31	*T. angustifolia*
COTL6	52.72	-1.37	*T. latifolia*
CRR6	47.68	22.46	*T. angustifolia*
CS3_TA	44.08	4.79	*T. laxmannii*
CY1ATL	52.16	4.50	*T. latifolia*
CY6ATA	51.43	-0.11	*T. angustifolia*
CY7ATA	51.43	-0.11	*T. angustifolia*
CY7BTA	51.43	-0.11	*T. angustifolia*
CYTZ_4ATD	-6.79	39.21	*T. domingensis*
DAB16TA	48.20	26.59	*T. angustifolia*
E74B	45.03	-63.50	*T. angustifolia*
E91C	45.03	-63.50	*T. angustifolia*
EDD_44_1	47.37	-68.33	*T. latifolia*
EDD_67_1	47.37	-68.33	*T. latifolia*
EDW_2_15	47.37	-68.33	*T. latifolia*
EDW_3_6	47.37	-68.33	*T. latifolia*
EL14_TA	52.44	5.85	*T. latifolia*
ELTA_01	52.44	5.85	*T. angustifolia*
ELTA_08	52.44	5.85	*T. angustifolia*
FRD47_2	45.96	-66.64	*T. latifolia*
FRTD_1	41.64	13.34	*T. domingensis*
GORE_3	43.68	-70.44	*T. latifolia*
G_II3	49.11	11.93	*T. latifolia*
HAD_3_1	44.60	-63.55	*T. angustifolia*
HAW_03_12	44.60	-63.55	*T. latifolia*
HAW_10_9	44.60	-63.55	*T. latifolia*
HM3TL	47.51	19.04	*T. latifolia*
HMTM1	47.51	19.04	*T. laxmannii*
HOR_6	48.14	26.51	*T. latifolia*
HUF_13TA	47.69	17.65	*T. angustifolia*
HUG_13TA	47.69	17.65	*T. angustifolia*
HUS_10TL	47.51	19.04	*T. latifolia*

HUS_TA1	47.51	19.04	*T. angustifolia*
HUS_TL1	47.51	19.04	*T. latifolia*
IC17TA	43.57	4.32	*T. angustifolia*
ICTD5	43.57	4.32	*T. angustifolia*
IP2	44.46	-64.32	*T. latifolia*
IR_25	44.97	-64.06	*T. latifolia*
IR_36	44.97	-64.06	*T. latifolia*
IR_39	44.97	-64.06	*T. latifolia*
IR_41	44.97	-64.06	*T. latifolia*
KL14_TL	46.64	14.31	*T. latifolia*
KL1TL	46.64	14.31	*T. latifolia*
KL28_TL	46.64	14.31	*T. latifolia*
KL4_TA	46.64	14.31	*T. laxmannii*
KL5_TX	46.64	14.31	*T. laxmannii*
KL_TA1	46.64	14.31	*T. laxmannii*
KL_TA3	46.64	14.31	*T. laxmannii*
KL_TH1	46.64	14.31	*T. latifolia*
LIETD_01	42.21	2.61	*T. domingensis*
LIETD_12	42.21	2.61	*T. domingensis*
LiETL_11	42.21	2.61	*T. latifolia*
M23	45.08	-64.49	*T. latifolia*
M30	45.08	-64.49	*T. latifolia*
M37	45.08	-64.49	*T. latifolia*
M53	45.08	-64.49	*T. latifolia*
M85	45.08	-64.49	*T. latifolia*
MBNK_2_8	51.51	-97.00	*T. latifolia*
MOD_01_0	46.98	-70.55	*T. latifolia*
MOD_02_1	46.98	-70.55	*T. latifolia*
MOD_04_1	46.98	-70.55	*T. latifolia*
MOD_05_2	46.98	-70.55	*T. latifolia*
MOD_07_0	46.98	-70.55	*T. angustifolia*
MOD_08_1	46.98	-70.55	*T. latifolia*
MOD_14_0	46.98	-70.55	*T. latifolia*
MOD_14_3	46.98	-70.55	*T. angustifolia*
MOD_15_1	46.98	-70.55	*T. latifolia*
MOD_16_1	46.98	-70.55	*T. latifolia*
MOD_17_1	46.98	-70.55	*T. latifolia*
MOD_18_0	46.98	-70.55	*T. latifolia*
MOD_25_2	46.98	-70.55	*T. latifolia*
MOD_26_2	46.98	-70.55	*T. latifolia*
MOD_29_1	46.98	-70.55	*T. latifolia*
MOD_35_3	46.98	-70.55	*T. latifolia*
MOD_45_2	46.98	-70.55	*T. latifolia*
MOD_47_1	46.98	-70.55	*T. angustifolia*
MOD_54_0	46.98	-70.55	*T. latifolia*
MOD_56_1	46.98	-70.55	*T. angustifolia*
MOD_57_0	46.98	-70.55	*T. latifolia*

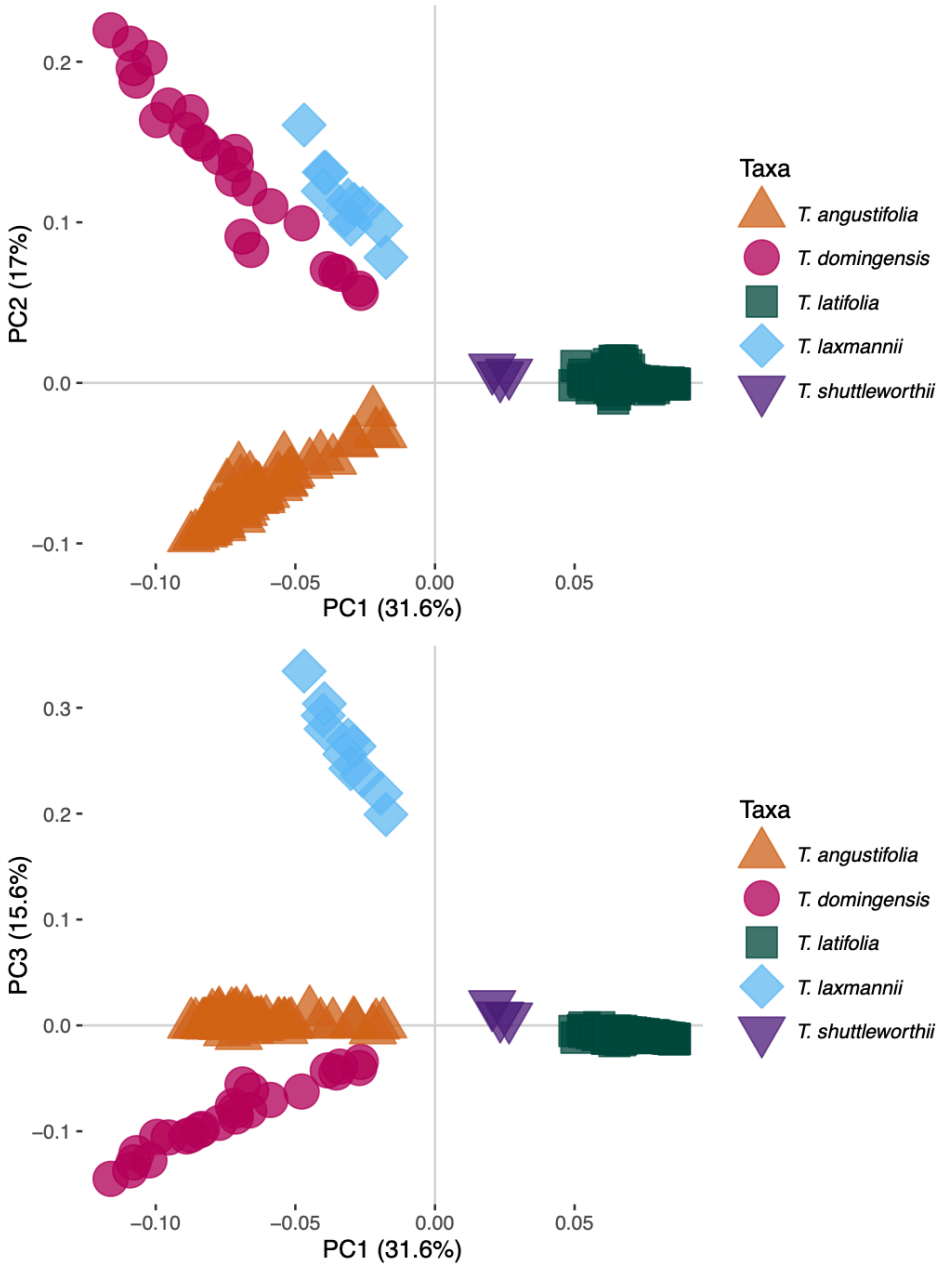
NBU_6	42.53	2.83	*T. domingensis*
ORP_TI	43.28	-2.13	*T. latifolia*
OR_TA4	43.28	-2.13	*T. domingensis*
OR_TI2	43.28	-2.13	*T. latifolia*
OR_TL5	43.28	-2.13	*T. latifolia*
P4	45.41	-64.33	*T. latifolia*
P6	45.41	-64.33	*T. latifolia*
P9	45.41	-64.33	*T. latifolia*
PA2	33.19	-112.15	*T. domingensis*
PIW_01_0	43.84	-79.10	*T. angustifolia*
PIW_04_6	43.84	-79.10	*T. angustifolia*
PIW_06_6	43.84	-79.10	*T. angustifolia*
PIW_07_1	43.84	-79.10	*T. angustifolia*
PIW_09_1	43.84	-79.10	*T. angustifolia*
PIW_10_2	43.84	-79.10	*T. angustifolia*
PIW_13_0	43.84	-79.10	*T. angustifolia*
PIW_14_3	43.84	-79.10	*T. angustifolia*
PIW_15_3	43.84	-79.10	*T. angustifolia*
PIW_16_2	43.84	-79.10	*T. angustifolia*
PIW_20_6	43.84	-79.10	*T. angustifolia*
PIW_21_3	43.84	-79.10	*T. angustifolia*
PIW_22_6	43.84	-79.10	*T. angustifolia*
PIW_23_3	43.84	-79.10	*T. angustifolia*
PIW_24_3	43.84	-79.10	*T. angustifolia*
PIW_25_3	43.84	-79.10	*T. angustifolia*
PWE19TL	51.62	-3.94	*T. latifolia*
RGT2	45.48	-74.30	*T. angustifolia*
SADT_03	45.90	-64.39	*T. angustifolia*
SAD_10_3	45.90	-64.39	*T. latifolia*
SAD_17_3	45.90	-64.39	*T. latifolia*
SAD_25_0	45.90	-64.39	*T. latifolia*
SCH9TA	47.65	26.22	*T. latifolia*
SP2_TA	52.41	4.68	*T. angustifolia*
TAKT_1	45.27	37.38	*T. angustifolia*
TAKT_2	45.27	37.38	*T. angustifolia*
TALD_1	55.73	37.61	*T. angustifolia*
TAVG_1	55.32	40.37	*T. angustifolia*
TAVG_2	55.32	40.37	*T. angustifolia*
TA_01_F	26.56	-81.95	*T. latifolia*
TB7	44.39	-64.25	*T. latifolia*
TDD_04	43.09	47.46	*T. domingensis*
TDD_08	43.09	47.46	*T. domingensis*
TDD_09	43.09	47.46	*T. domingensis*
TDD_15	43.09	47.46	*T. domingensis*
TDD_16	43.09	47.46	*T. latifolia*
TDD_17	43.09	47.46	*T. latifolia*
TDD_18	43.09	47.46	*T. domingensis*

TDD_22	43.09	47.46	*T. domingensis*
TDD_30	43.09	47.46	*T. domingensis*
TDD_32	43.09	47.46	*T. domingensis*
TDD_33	43.09	47.46	*T. domingensis*
TDD_35	43.09	47.46	*T. latifolia*
TDD_36	43.09	47.46	*T. latifolia*
TDD_37	43.09	47.46	*T. latifolia*
TDD_39	43.09	47.46	*T. latifolia*
TDD_40	43.09	47.46	*T. domingensis*
TDD_41	43.09	47.46	*T. latifolia*
TDD_42	43.09	47.46	*T. latifolia*
TDD_43	43.09	47.46	*T. angustifolia*
TDD_44	43.09	47.46	*T. domingensis*
TDD_46	43.09	47.46	*T. domingensis*
TDD_47	43.09	47.46	*T. domingensis*
TDD_49	43.09	47.46	*T. angustifolia*
TDD_50	43.09	47.46	*T. domingensis*
TDD_51	43.09	47.46	*T. domingensis*
TDD_52	43.09	47.46	*T. domingensis*
TDD_53	43.09	47.46	*T. domingensis*
TDD_54	43.09	47.46	*T. domingensis*
TLAX_1	43.35	47.24	*T. laxmannii*
TLAX_2	43.35	47.24	*T. laxmannii*
TLAX_6	41.63	48.35	*T. laxmannii*
TLAX_7	41.63	48.35	*T. laxmannii*
TLIK_01	45.09	147.89	*T. latifolia*
TLIK_02	45.09	147.89	*T. latifolia*
TLIK_03	45.09	147.89	*T. latifolia*
TLIK_04	45.09	147.89	*T. latifolia*
TLIK_06	45.09	147.89	*T. latifolia*
TLIK_11	45.09	147.89	*T. latifolia*
TLIK_14	45.09	147.89	*T. latifolia*
TLIK_15	45.09	147.89	*T. latifolia*
TLIK_16	45.09	147.89	*T. latifolia*
TLIK_24	45.09	147.89	*T. latifolia*
TLKL_2	45.23	39.07	*T. laxmannii*
TLMK_1	45.21	38.89	*T. latifolia*
TLMK_2	45.21	38.89	*T. latifolia*
TLPK_1	45.09	147.89	*T. latifolia*
TLSK_01	47.06	142.53	*T. latifolia*
TLSK_02	47.06	142.53	*T. latifolia*
TLSK_03	47.06	142.53	*T. latifolia*
TLSK_04	47.06	142.53	*T. latifolia*
TLSK_11	47.06	142.53	*T. latifolia*
TLSK_12	47.06	142.53	*T. latifolia*
TMO2_7	46.09	-64.78	*T. latifolia*
TSKL_1	43.85	39.71	*T. shuttleworthii*

TSKL_2	43.85	39.71	*T. shuttleworthii*
TSKL_3	43.85	39.71	*T. shuttleworthii*
TXKT_1	45.29	37.41	*T. angustifolia*
TXKT_2	45.29	37.41	*T. angustifolia*
TXKT_3	45.29	37.41	*T. angustifolia*
TXKT_4	45.29	37.41	*T. angustifolia*
TXKT_6	45.29	37.41	*T. angustifolia*
TXVG_1	55.32	40.37	*T. angustifolia*
TXVG_2	55.32	40.37	*T. angustifolia*
VFW6	49.63	-125.40	*T. latifolia*
Vikram_25	44.30	-78.32	*T. angustifolia*
Vikram_33	44.30	-78.32	*T. angustifolia*
Vikram_73	44.30	-78.32	*T. angustifolia*
Vikram_76	44.30	-78.32	*T. latifolia*
Vikram_86	44.30	-78.32	*T. latifolia*
Vikram_94	44.30	-78.32	*T. angustifolia*
Vikram_95	44.30	-78.32	*T. latifolia*
WBD20_1	44.28	-84.23	*T. angustifolia*
WBD_27_4	44.28	-84.23	*T. angustifolia*
WBD_28_3	44.28	-84.23	*T. angustifolia*
WBD_32_1	44.28	-84.23	*T. angustifolia*
WBD_33_1	44.28	-84.23	*T. angustifolia*
WBW5_4	44.28	-84.23	*T. latifolia*

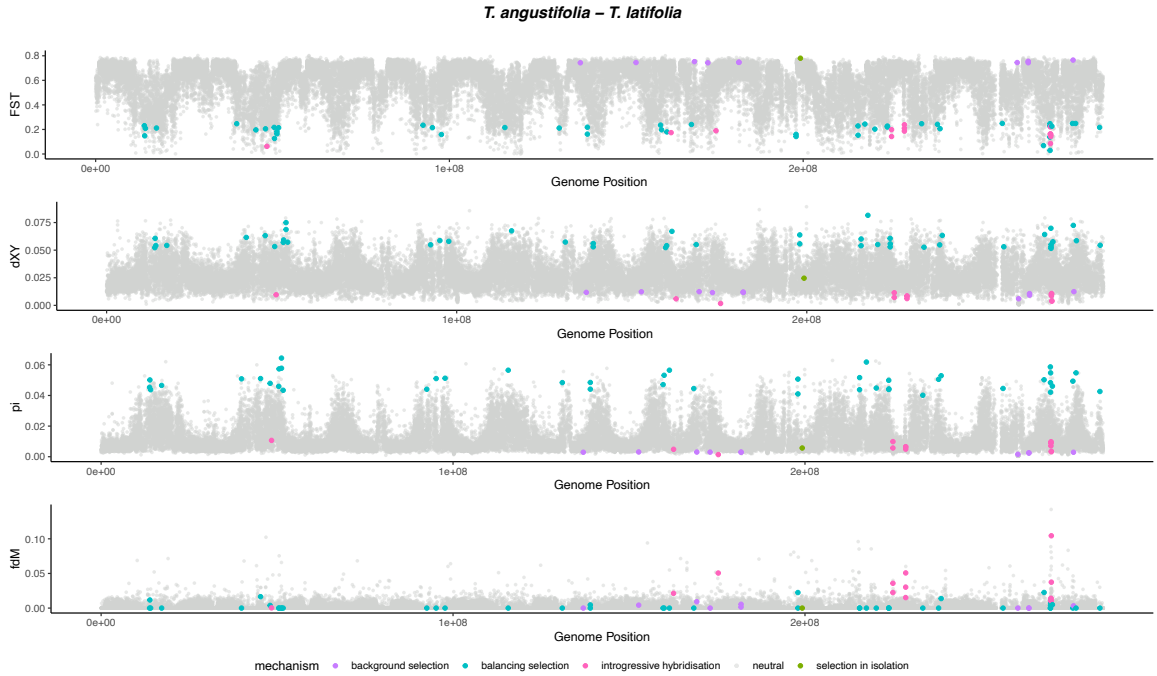
Supplementary Table S3.2. Combinations of ingroup-outgroup (P1) recipient (P2), and donor species (P3) used during the ABBA BABA test to look for introgressive hybridisation between four *Typha* spp.; *T. laxmannii* was established as the outgroup species for all comparisons.

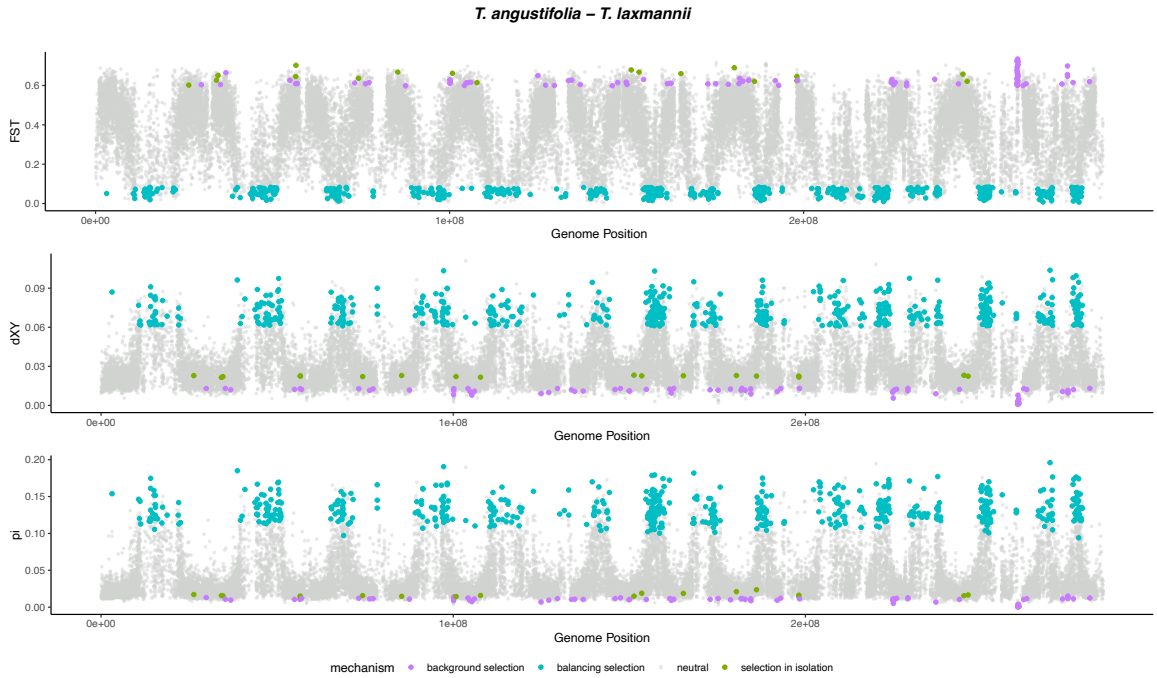
P1	**P2**	**P3**
T. angustifolia	*T. domingensis*	*T. latifolia*
T. angustifolia	*T. domingensis*	*T. shuttleworthii*
T. domingensis	*T. angustifolia*	*T. latifolia*
T. domingensis	*T. angustifolia*	*T. shuttleworthii*
T. shuttleworthii	*T. latifolia*	*T. angustifolia*
T. shuttleworthii	*T. latifolia*	*T. domingensis*

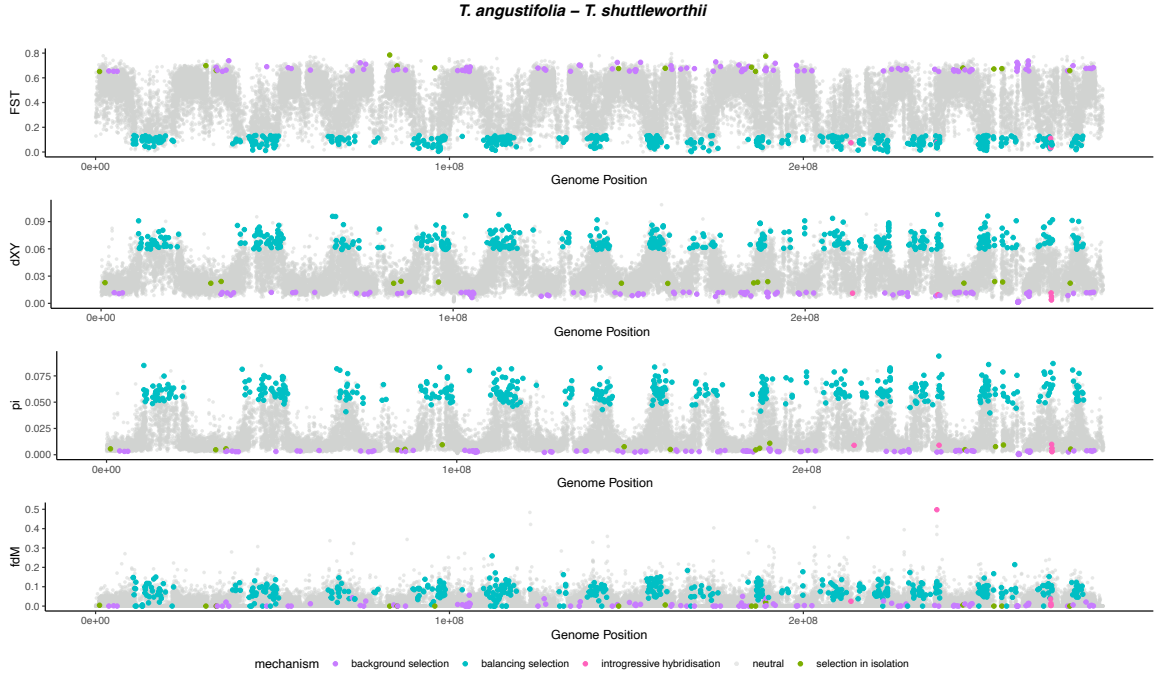


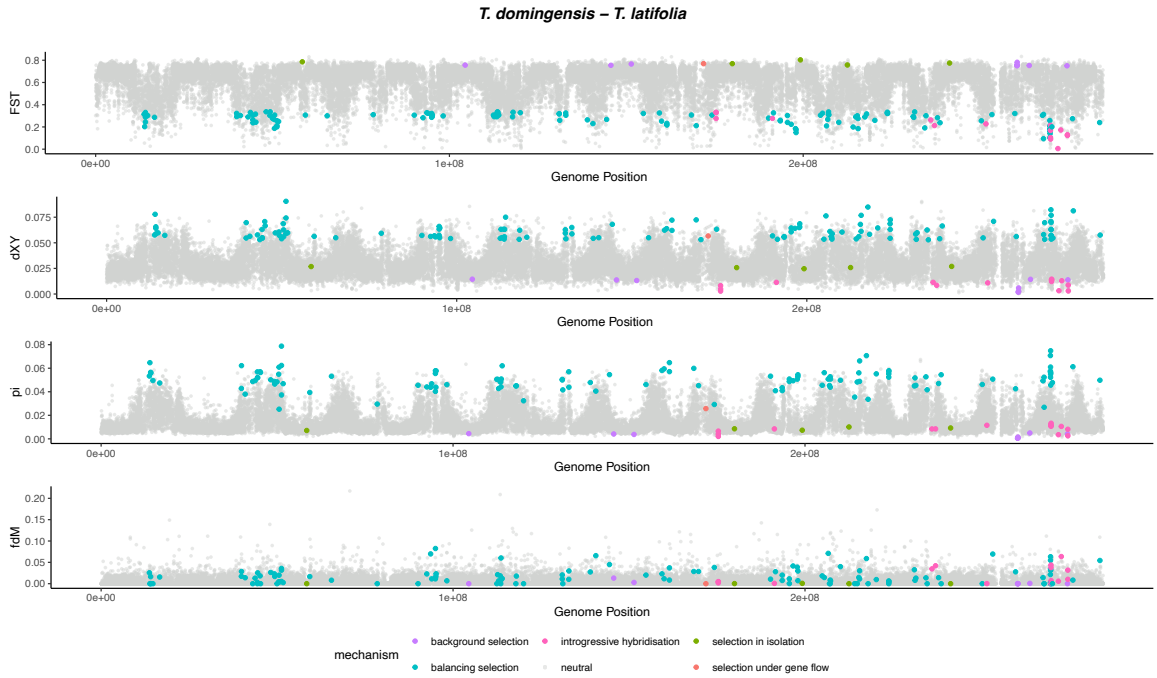
Supplementary Figure S3.1. Principal component analysis for PC1-PC2 and PC1-PC3. Individual shapes represent individuals, and shape forms and colour codes represent species, as in the box.

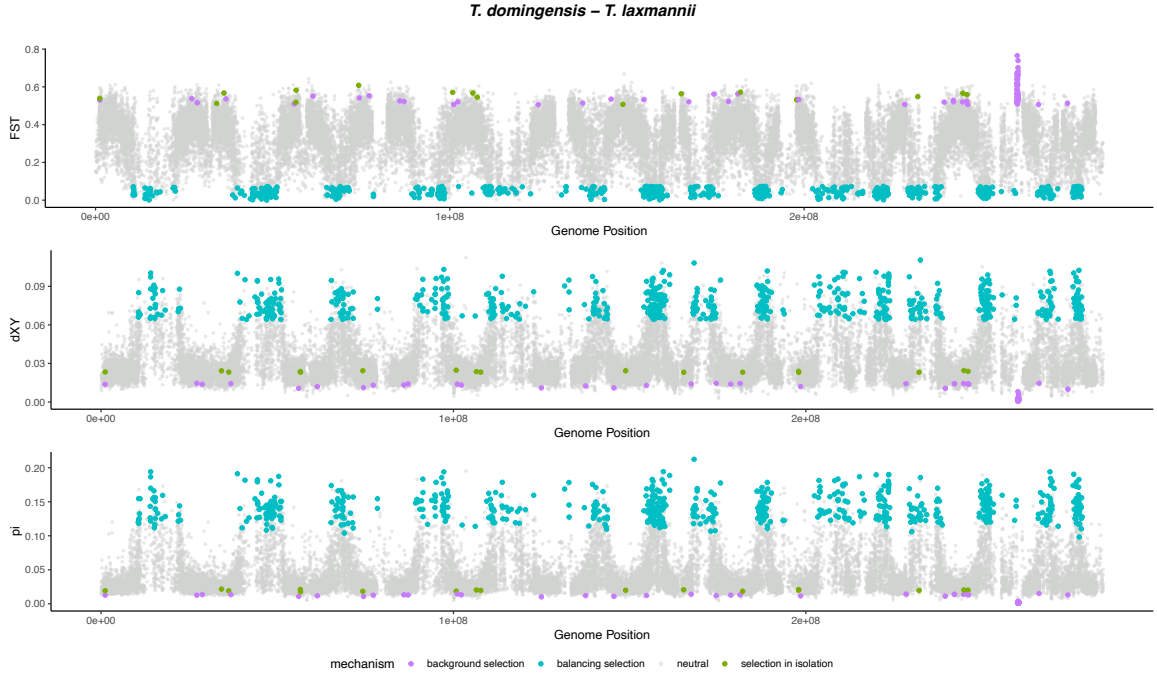

T. angustifolia – T. domingensis
FST
dXY
pi
Genome Position
0e+00
1e+08
2e+08
mechanism
background selection
balancing selection
neutral
selection in isolation


T. angustifolia – T. latifolia
FST
dXY
pi
fdM
Genome Position
0e+00
1e+08
2e+08
mechanism
background selection
balancing selection
introgressive hybridisation
neutral
selection in isolation

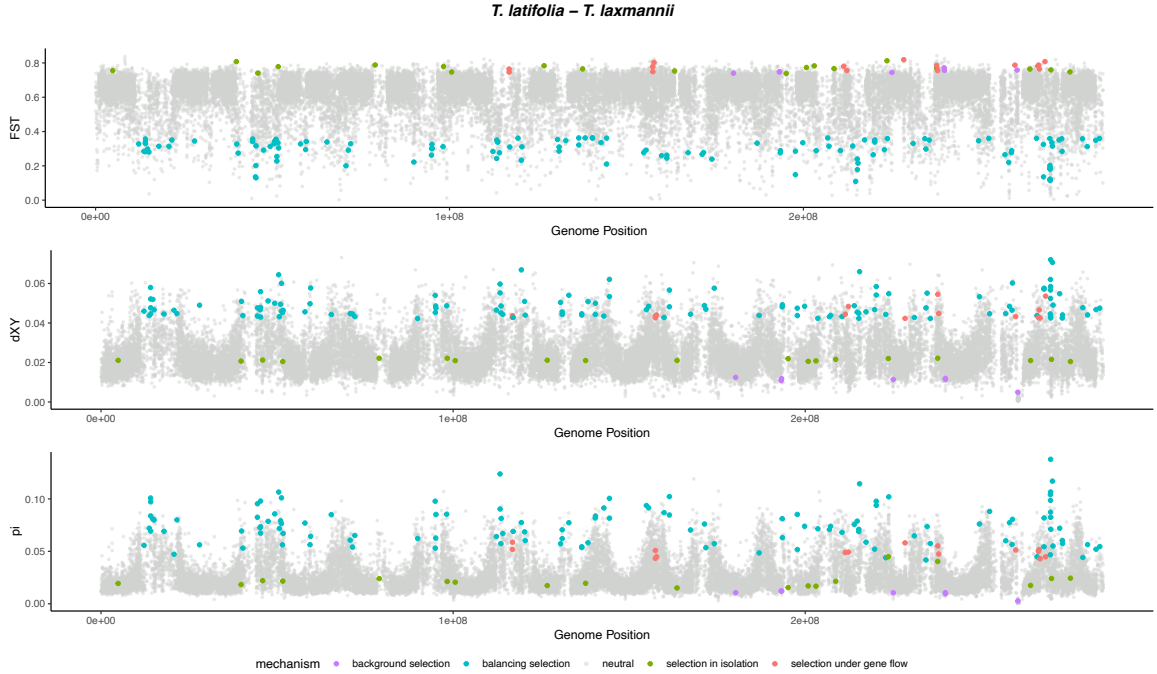

T. angustifolia – T. laxmannii
FST
dXY
pi
Genome Position
0e+00
1e+08
2e+08
mechanism
background selection
balancing selection
neutral
selection in isolation


T. angustifolia – T. shuttleworthii
FST
dXY
pi
fdM
Genome Position
0e+00
1e+08
2e+08
mechanism
background selection
balancing selection
introgressive hybridisation
neutral
selection in isolation


T. domingensis – T. latifolia
FST
dXY
pi
fdM
Genome Position
0e+00
1e+08
2e+08
mechanism
background selection
introgressive hybridisation
selection in isolation
balancing selection
neutral
selection under gene flow


T. domingensis – T. laxmannii
FST
dXY
pi
Genome Position
0e+00
1e+08
2e+08
mechanism
background selection
balancing selection
neutral
selection in isolation


T. domingensis – T. shuttleworthii
FST
dXY
pi
fdM
Genome Position
0e+00
1e+08
2e+08
mechanism
background selection
balancing selection
neutral
potential introgression
selection in isolation


T. latifolia – T. laxmannii
FST
dXY
pi
Genome Position
0e+00
1e+08
2e+08
mechanism
background selection
balancing selection
neutral
selection in isolation
selection under gene flow

Supplementary Figures S3.2 to S3.11. Genomic landscapes of divergence between five *Typha* species. Results from *Role of selection and introgressive hybridisation on species' divergence*. Islands and valleys of divergence (5 kb) consistent with alternative types of selection are coloured according to the legend.

Supplementary Figures S3.12 and S3.13. Genomic landscapes of divergence between *Typha angustifolia* and *T. latifolia* split by continent (North America and Europe). Islands and valleys of divergence (5 kb) consistent with alternative types of selection are coloured according to the legend.

Supplementary materials for Chapter 4

Supplementary Table S4.1. Identifiers, species memberships, approximate geographic coordinates, and continent of the samples collected in this study.

Sampling ID	Species	Latitude	Longitude	Continent
B12	*T. × glauca*	45.08	-64.49	North America
BRT2B	*T. angustifolia*	44.74	-63.24	North America
BU14	*T. × glauca*	42.35	-3.69	Europe
C05TL	*T. latifolia*	52.72	-1.37	Europe
CA13_TL	*T. latifolia*	44.85	7.71	Europe
CA3TL	*T. latifolia*	47.68	22.46	Europe
CC1_TL	*T. latifolia*	50.77	2.31	Europe
CC2_TL	*T. latifolia*	50.77	2.31	Europe
CIUU13TA	*T. angustifolia*	43.76	24.93	Europe
CL10TA	*T. angustifolia*	50.77	2.31	Europe
COTL6	*T. latifolia*	52.72	-1.37	Europe
CRR6	*T. angustifolia*	47.68	22.46	Europe
CY1ATL	*T. latifolia*	52.16	4.50	Europe
CY6ATA	*T. angustifolia*	51.43	-0.11	Europe
CY7ATA	*T. angustifolia*	51.43	-0.11	Europe
CY7BTA	*T. angustifolia*	51.43	-0.11	Europe
DAB16TA	*T. angustifolia*	48.20	26.59	Europe
E74B	*T. angustifolia*	45.03	-63.50	North America
E91C	*T. angustifolia*	45.03	-63.50	North America
EDD_44_1	*T. latifolia*	47.37	-68.33	North America
EDD_67_1	*T. latifolia*	47.37	-68.33	North America
EDW_2_15	*T. latifolia*	47.37	-68.33	North America
EDW_3_6	*T. latifolia*	47.37	-68.33	North America
EL14_TA	*T. latifolia*	52.44	5.85	Europe
ELTA_01	*T. angustifolia*	52.44	5.85	Europe
ELTA_08	*T. angustifolia*	52.44	5.85	Europe
FRD47_2	*T. latifolia*	45.96	-66.64	North America
GORE_3	*T. latifolia*	43.68	-70.44	North America
G_II3	*T. latifolia*	49.11	11.93	Europe
HAD_3_1	*T. angustifolia*	44.60	-63.55	North America
HAW_03_12	*T. latifolia*	44.60	-63.55	North America
HAW_10_9	*T. latifolia*	44.60	-63.55	North America
HM3TL	*T. latifolia*	47.51	19.04	Europe
HOR_6	*T. latifolia*	48.14	26.51	Europe
HUF_13TA	*T. angustifolia*	47.69	17.65	Europe
HUG_13TA	*T. angustifolia*	47.69	17.65	Europe
HUS_10TL	*T. latifolia*	47.51	19.04	Europe
HUS_TA1	*T. angustifolia*	47.51	19.04	Europe
HUS_TL1	*T. latifolia*	47.51	19.04	Europe
IC17TA	*T. angustifolia*	43.57	4.32	Europe
ICTD5	*T. angustifolia*	43.57	4.32	Europe
IP2	*T. latifolia*	44.46	-64.32	North America
IR_01	*T. × glauca*	44.97	-64.06	North America

IR_105	*T. × glauca*	44.97	-64.06	North America
IR_11	*T. angustifolia*	44.97	-64.06	North America
IR_119	*T. × glauca*	44.97	-64.06	North America
IR_25	*T. latifolia*	44.97	-64.06	North America
IR_36	*T. latifolia*	44.97	-64.06	North America
IR_39	*T. latifolia*	44.97	-64.06	North America
IR_41	*T. latifolia*	44.97	-64.06	North America
KL14_TL	*T. latifolia*	46.64	14.31	Europe
KL1TL	*T. latifolia*	46.64	14.31	Europe
KL28_TL	*T. latifolia*	46.64	14.31	Europe
KL_TH1	*T. latifolia*	46.64	14.31	Europe
LiETL_11	*T. latifolia*	42.21	2.61	Europe
M114	*T. × glauca*	45.08	-64.49	North America
M23	*T. latifolia*	45.08	-64.49	North America
M30	*T. latifolia*	45.08	-64.49	North America
M37	*T. latifolia*	45.08	-64.49	North America
M53	*T. latifolia*	45.08	-64.49	North America
M85	*T. latifolia*	45.08	-64.49	North America
MOD_01_0	*T. latifolia*	46.98	-70.55	North America
MOD_02_1	*T. latifolia*	46.98	-70.55	North America
MOD_04_1	*T. latifolia*	46.98	-70.55	North America
MOD_05_2	*T. latifolia*	46.98	-70.55	North America
MOD_07_0	*T. angustifolia*	46.98	-70.55	North America
MOD_08_1	*T. latifolia*	46.98	-70.55	North America
MOD_09_0	*T. angustifolia*	46.98	-70.55	North America
MOD_14_0	*T. latifolia*	46.98	-70.55	North America
MOD_14_3	*T. angustifolia*	46.98	-70.55	North America
MOD_15_1	*T. latifolia*	46.98	-70.55	North America
MOD_16_1	*T. latifolia*	46.98	-70.55	North America
MOD_17_1	*T. latifolia*	46.98	-70.55	North America
MOD_18_0	*T. latifolia*	46.98	-70.55	North America
MOD_25_2	*T. latifolia*	46.98	-70.55	North America
MOD_26_2	*T. latifolia*	46.98	-70.55	North America
MOD_29_1	*T. latifolia*	46.98	-70.55	North America
MOD_33_0	*T. × glauca*	46.98	-70.55	North America
MOD_35_3	*T. latifolia*	46.98	-70.55	North America
MOD_37_1	*T. × glauca*	46.98	-70.55	North America
MOD_38_1	*T. × glauca*	46.98	-70.55	North America
MOD_39_0	*T. × glauca*	46.98	-70.55	North America
MOD_40_0	*T. × glauca*	46.98	-70.55	North America
MOD_42_3	*T. × glauca*	46.98	-70.55	North America
MOD_45_2	*T. latifolia*	46.98	-70.55	North America
MOD_47_1	*T. angustifolia*	46.98	-70.55	North America
MOD_50_1	*T. × glauca*	46.98	-70.55	North America
MOD_52_0	*T. × glauca*	46.98	-70.55	North America
MOD_54_0	*T. latifolia*	46.98	-70.55	North America
MOD_55_0	*T. × glauca*	46.98	-70.55	North America

MOD_56_1	*T. angustifolia*	46.98	-70.55	North America
MOD_57_0	*T. latifolia*	46.98	-70.55	North America
MOD_58_0	*T. × glauca*	46.98	-70.55	North America
MOD_59_1	*T. × glauca*	46.98	-70.55	North America
MOD_60_0	*T. × glauca*	46.98	-70.55	North America
MOD_61_0	*T. × glauca*	46.98	-70.55	North America
MOD_62_1	*T. × glauca*	46.98	-70.55	North America
MOD_63_0	*T. × glauca*	46.98	-70.55	North America
ORP_TI	*T. latifolia*	43.28	-2.13	Europe
OR_TI2	*T. latifolia*	43.28	-2.13	Europe
OR_TL5	*T. latifolia*	43.28	-2.13	Europe
P4	*T. latifolia*	45.41	-64.33	North America
P6	*T. latifolia*	45.41	-64.33	North America
P9	*T. latifolia*	45.41	-64.33	North America
PID_04_3	*T. × glauca*	43.84	-79.10	North America
PID_08_5	*T. × glauca*	43.84	-79.10	North America
PID_09_0	*T. × glauca*	43.84	-79.10	North America
PID_10_1	*T. × glauca*	43.84	-79.10	North America
PIW_01_0	*T. angustifolia*	43.84	-79.10	North America
PIW_04_6	*T. angustifolia*	43.84	-79.10	North America
PIW_06_6	*T. angustifolia*	43.84	-79.10	North America
PIW_07_1	*T. angustifolia*	43.84	-79.10	North America
PIW_09_1	*T. angustifolia*	43.84	-79.10	North America
PIW_10_2	*T. angustifolia*	43.84	-79.10	North America
PIW_12_3	*T. × glauca*	43.84	-79.10	North America
PIW_13_0	*T. angustifolia*	43.84	-79.10	North America
PIW_14_3	*T. angustifolia*	43.84	-79.10	North America
PIW_15_3	*T. angustifolia*	43.84	-79.10	North America
PIW_16_2	*T. angustifolia*	43.84	-79.10	North America
PIW_20_6	*T. angustifolia*	43.84	-79.10	North America
PIW_21_3	*T. angustifolia*	43.84	-79.10	North America
PIW_22_6	*T. angustifolia*	43.84	-79.10	North America
PIW_23_3	*T. angustifolia*	43.84	-79.10	North America
PIW_24_3	*T. angustifolia*	43.84	-79.10	North America
PIW_25_3	*T. angustifolia*	43.84	-79.10	North America
PWE19TL	*T. latifolia*	51.62	-3.94	Europe
RGT2	*T. angustifolia*	45.48	-74.30	North America
SADT_03	*T. angustifolia*	45.90	-64.39	North America
SAD_10_3	*T. latifolia*	45.90	-64.39	North America
SAD_17_3	*T. latifolia*	45.90	-64.39	North America
SAD_25_0	*T. latifolia*	45.90	-64.39	North America
SCH9TA	*T. latifolia*	47.65	26.22	Europe
SP2_TA	*T. angustifolia*	52.41	4.68	Europe
SR2_TA	*T. × glauca*	52.41	4.68	Europe
TA_01	*T. angustifolia*	51.48	0.61	Europe
TB7	*T. latifolia*	44.39	-64.25	North America
TMO2_7	*T. latifolia*	46.09	-64.78	North America

Vikram_202	*T.* × *glauca*	44.30	-78.32	North America
Vikram_237	*T.* × *glauca*	44.30	-78.32	North America
Vikram_25	*T. angustifolia*	44.30	-78.32	North America
Vikram_28	*T.* × *glauca*	44.30	-78.32	North America
Vikram_33	*T. angustifolia*	44.30	-78.32	North America
Vikram_73	*T. angustifolia*	44.30	-78.32	North America
Vikram_76	*T. latifolia*	44.30	-78.32	North America
Vikram_86	*T. latifolia*	44.30	-78.32	North America
Vikram_94	*T. angustifolia*	44.30	-78.32	North America
Vikram_95	*T. latifolia*	44.30	-78.32	North America
WBD20_1	*T. angustifolia*	44.28	-84.23	North America
WBD_22_5	*T.* × *glauca*	44.28	-84.23	North America
WBD_27_4	*T. angustifolia*	44.28	-84.23	North America
WBD_28_3	*T. angustifolia*	44.28	-84.23	North America
WBD_29_2	*T.* × *glauca*	44.28	-84.23	North America
WBD_32_1	*T. angustifolia*	44.28	-84.23	North America
WBD_33_1	*T. angustifolia*	44.28	-84.23	North America
WBD_34_1	*T.* × *glauca*	44.28	-84.23	North America
WBD_36_2	*T.* × *glauca*	44.28	-84.23	North America
WBW5_4	*T. latifolia*	44.28	-84.23	North America
WBW_14_4	*T.* × *glauca*	44.28	-84.23	North America
WBW_16_2	*T.* × *glauca*	44.28	-84.23	North America
WBW_18_6	*T.* × *glauca*	44.28	-84.23	North America

Supplementary Table S4.2. Prior bounds of the divergence times and effective population sizes used during the reconstruction of the demographic and divergence histories of *T. angustifolia* and *T. latifolia* in North America and Europe.

	North America	**Europe**	***T. angustifolia* (North America) – *T. latifolia* (Europe)**	***T. angustifolia* (Europe) – *T. latifolia* (North America)**
Minimum effective size (N_e)	1,071	1,429	1,429	1,071
Minimum effective size (N_e)	608,929	652,036	608,929	652,036
Maximum divergence time (in generations)	864,285	801,482	834,821	830,946
Maximum divergence time (in generations)	1,728,571	1,602,965	1,669,642	1,661,892

Supplementary Table S4.3. Likelihood of the different scenarios tested during the reconstruction of the demographic history of *T. angustifolia* and *T. latifolia* in North America and Europe. Scenarios with the highest likelihood are denoted in **bold**. AM = Ancestral migration; IM = Isolation–Migration; SC = Secondary contact; SI = Strict Isolation; 1M = Rates of interspecific gene flow remained constant over time; 2M = Rates of interspecific gene flow changed over time; 1N = Effective sizes (N_e) remained constant over time; 2N = Effective sizes (N_e) changed over time.

	North America	**Europe**	***T. angustifolia* (North America) – *T. latifolia* (Europe)**	***T. angustifolia* (Europe) – *T. latifolia* (North America)**
AM_1M_1N	0.00131801	0.00275322	0.00158906	0.0007579
AM_1M_2N	0.01451122	0.01326868	0.00758491	0.01287907
AM_2M_1N	0.00151503	0.0095897	0.01217523	0.00171429
AM_2M_2N	0.01475842	0.01662897	0.00881292	0.01834401
IM_1M_1N	0.04620111	0.04382832	0.0344655	0.0420938
IM_1M_2N	0.12083723	0.10250842	0.07366645	0.09782658
IM_2M_1N	0.08616011	0.09540489	0.08519177	0.10659093
IM_2M_2N	**0.21147222**	0.16850417	0.1730375	**0.18919861**
SC_1M_1N	0.08448302	0.09026685	0.10169659	0.07249265
SC_1M_2N	0.16397194	0.15623251	0.1673672	0.18099716
SC_2M_1N	0.08190043	0.10936329	0.09894899	0.10191291
SC_2M_2N	0.16227781	**0.18477438**	**0.23111132**	0.16810195
SI_1N	0.0003016	0.00097208	0.00126732	0.00030913
SI_2N	0.01029183	0.00590454	0.00308525	0.00678103

Supplementary Table S4.4 Barrier loci between *T. angustifolia* and *T. latifolia* in Europe and between species from different continents. No barrier loci were identified in North America. Unless otherwise indicated in the observations, barrier loci were found in Europe. BLAST = Genes predicted by BLAST (Camacho et al. 2009).

Chr	Coords.	Annotation/Predicted protein	Reference	Observations
1	6585000 – 6590000	At4g11680	(Stone et al. 2005)	–
1	12400000 – 12405000	Cinv1	(Barratt et al. 2009)	Positive selection in *T. latifolia*
1	13925000 – 13930000	AP2-like ethylene-responsive transcription factor AIL5	BLAST	F_{ST} outlier
1	22255000 – 22260000	Uncharacterized protein	BLAST	F_{ST} outlier
1	32070000 – 32075000	Serine/threonine-protein kinase D6PK-like	BLAST	–
2	65000 – 70000	Dof zinc finger protein DOF2.1-like	BLAST	F_{ST} outlier
2	13045000 – 13050000	Ccb2	(Lyska et al. 2007)	F_{ST} outlier
2	16240000 – 16245000	Ugt80a2	(DeBolt et al. 2009)	F_{ST} outlier
2	17600000 – 17605000	Uncharacterized protein	BLAST	–
2	17685000 – 17690000	Uncharacterized protein	BLAST	–
2	21520000 – 21525000	Uncharacterized protein	BLAST	–
2	25195000 – 25200000	Casp1	(Roppolo et al. 2011)	–
3	650000 – 655000	CBBY-like protein	BLAST	F_{ST} outlier
3	8840000 – 8845000	Glutathione gamma-glutamylcysteinyltransferase 1-like	BLAST	–
3	20660000 – 20665000	Transcription factor KUA1-like	BLAST	–
3	22825000 – 22830000	Uncharacterized protein	BLAST	–

3	23070000 – 23075000	Xyloglucan endotransglucosylase protein 6-like	BLAST	–
4	1400000 – 1405000	F-box/LRR-repeat protein At3g26922-like	BLAST	–
4	10560000 – 10565000	Polyol transporter 5-like	BLAST	–
4	23330000 – 23335000	UPF0481 protein At3g47200 – like	BLAST	F_{ST} and d_{XY} outlier
5	8975000 – 8980000	Uncharacterized protein	BLAST	–
6	820000 – 825000	Uncharacterized protein	BLAST	–
7	3645000 – 3650000	–	–	F_{ST} outlier
7	12275000 – 12280000	Probable disease resistance protein At4g14610	BLAST	–
7	12320000 – 12325000	Uncharacterized protein	BLAST	–
8	860000 – 865000	Uncharacterized protein	BLAST	–
8	3230000 – 3235000	Uncharacterized protein	BLAST	–
9	11845000 – 11850000	Pair2	(Nonomura et al. 2006)	–
9	13585000 – 13590000	Uncharacterized protein	BLAST	–
10	6130000 – 6135000	–	–	–
11	725000 – 730000	SWI/SNF complex component SNF12 homolog	BLAST	–
11	1975000 – 1980000	Uncharacterized lncRNA	BLAST	–
11	4780000 – 4785000	Uncharacterized lncRNA	BLAST	Only in *T. angustifolia* (North America) vs. *T. latifolia* (Europe)
11	5550000 – 5555000	–	–	Both in (i) Europe and (ii) *T. angustifolia* (Europe) vs. *T. latifolia* (North America)
11	5880000 – 5885000	Uncharacterized lncRNA	BLAST	Both in (i) Europe and (ii) *T. angustifolia* (North America) vs. *T. latifolia* (Europe)
11	10935000 – 10940000	Disease resistance protein RGA2-like	BLAST	–

12	7595000 – 7600000	Tubulin beta-3 chain	BLAST	–
13	4645000 – 4650000	Gamma-tubulin complex component 2 LAT	BLAST	–
13	9235000 – 9240000	Pdcd2	(Baron et al. 2010)	–
13	11910000 – 11925000	Iaa19	(Liscum and Reed 2002)	Purifying selection between species
13	12670000 – 12675000	Almt12	(Sasaki et al. 2010)	–
14	70000 – 75000	ABC transporter C family member 5-like	BLAST	–
14	5990000 – 5995000	Receptor-like protein EIX2	BLAST	–
14	12090000 – 12095000	Uncharacterized protein (LOC140763314), mRNA	BLAST	Only in *T. angustifolia* (North America) vs. *T. latifolia* (Europe)
15	9910000 – 9915000	Uncharacterized lncRNA	BLAST	–
15	10745000 – 10750000	Phosphatidylinositol/phosphatidylcholine transfer protein SFH9-like	BLAST	–
15	11705000 – 11710000	Probable E3 ubiquitin-protein ligase ARI8	BLAST	F_{ST} outlier