Running title: Allopatric speciation in cattails: Genomic landscapes of divergence across *Typha* spp. suggest balancing selection, introgressions, and the absence of adaptive divergence

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Abstract

Determining the evolutionary forces which result in the accumulation of genetic divergence between closely related species can yield important insights into speciation. Genetic divergence can be understood by two broad non-mutually exclusive frameworks: genetic drift under allopatry and natural selection under ecological divergence. Genetic regions that are highly differentiated or conserved between closely related species can be used to infer the types of selection driving speciation. We tested the role of selection in promoting species divergence in *Typha*, an old, widespread plant genus characterised by high levels of intra- and interspecific gene flow. Using genome-wide data, we scanned the genomes of multiple individuals from five *Typha* species to identify how selection influenced their levels of genetic divergence. We also tested whether past admixture or contemporary introgression events have contributed to the genetic variation within and among species. The genomic landscapes of divergence were predominantly neutral, including regions of exceptional differentiation, and we observed contemporary genetic introgressions with no evidence of historical hybridisation. The absence of adaptive divergence and historical hybridisation are consistent with patterns expected under allopatric speciation and genetic divergence driven primarily by drift, followed by range expansion and secondary contact leading to contemporary hybridisation and recent genetic introgressions. For all

species pairs, most genomic regions associated with adaptive divergence were valleys of balancing selection, in which the maintenance of ancestral polymorphisms causes reduced levels of divergence. Reproductive isolation among multiple *Typha* species remains incomplete, and balancing selection and recent introgressions have contributed to their standing genetic variation.

Introduction

Understanding how genetic divergence accumulates between species during the course of speciation is a key aspect of evolutionary biology (Wright, 1950; Hedrick, 2009; Seehausen *et al.*, 2014). Comparisons of congeneric species across different stages of divergence have shed light on the ways in which genomic barriers emerge and shift across the genome and the speciation continua (Irwin *et al.*, 2018; Shang *et al.*, 2023). Additionally, examining the amount of genetic diversity and divergence between related species has provided insights into the roles of standing genetic variation, new mutations, and introgression as sources of adaptive variation (Tigano and Friesen, 2016; Ravinet *et al.*, 2017; Wolf and Ellegren, 2017). Together, these help us to understand the processes that produce and preserve biological diversity (The Marie Curie SPECIATION Network, 2012; Abbott *et al.*, 2013).

Genetic divergence between species is expected to accumulate differently across the genome depending on the forces driving speciation and whether they occur in allopatry or sympatry (i.e., without or with gene flow) (Ellegren and Galtier, 2016). In allopatry, divergence is driven by drift across most loci (Savolainen et al., 2013; Sork, 2018; Wadgymar et al., 2022; e.g., Wu et al., 2015), and although adaptation can play a role in allopatric speciation, it is less prominent than when species diverge in sympatry (Hernández-Hernández *et al.*, 2021). Under sympatry, selection is

the primary driver of divergence (Pinho and Hey, 2010). Reproductive barriers in the form of barrier loci are expected to emerge more frequently or be stronger when divergence is driven by selection rather than by drift (Stukenbrock, 2014; Papadopulos *et al.*, 2019). In contrast, allopatric speciation can lead to subsequent interspecific hybridisation if species experience secondary contact following range expansion, as frequently seen in plant species (Mallet, 2005; Givnish, 2010).

Genome-wide sequencing has generated detailed patterns of genetic divergence between species across entire genomes (Quilodrán *et al.*, 2020), and can identify the roles of selection versus drift in speciation (Bock *et al.*, 2023). Genetic regions that are highly differentiated ("islands") or conserved ("valleys") between closely related species – compared to the genomic background – have been used to infer the types of selection driving speciation (Wolf and Ellegren, 2017). Four non-exclusive types of selection can be identified by scanning genomes and comparing levels of relative and absolute differentiation (F_{ST} and d_{XY}) and patterns of nucleotide diversity (π) between species (Irwin et al., 2018; Kessler et al., 2023; Shang et al., 2023). These are divergent selection (i) with and (ii) without gene flow, as expected under sympatry and allopatry, respectively; (iii) background selection in allopatry; and (iv) balancing selection, characterised as the maintenance of ancestral polymorphisms, either in sympatry or allopatry. While background selection is the null model when investigating genomic

divergence (Comeron, 2017), balancing selection has been widely identified across taxa (Guerrero and Hahn, 2017; Sendell-Price *et al.*, 2020; Ali *et al.*, 2024). Furthermore, incorporating introgression tests when scanning patterns of genetic divergence allows testing the role of interspecific gene flow as a source of genetic variation in hybridising species (Bay and Ruegg, 2017; Setter *et al.*, 2020).

We examined the genomic landscapes of speciation of five cattail species to test the role of selection as the driver of speciation in the genus *Typha* L. Cattails are rhizomatous perennial, monoecious, self-compatible, and wind-pollinated plants crucial to freshwater and brackish ecosystems (reviewed by Bansal et al., 2019). The genus ischaracterised by widespread distributions across every continent except Antarctica; high levels of intra- and interspecific gene flow (Pieper et al., 2020); and old divergence times (20 – 70 Ma; Bremer, 2000; Widanagama et al., 2022; Zhou et al., 2018). Three of the species we focussed on (*T. angustifolia* L., *T. domingensis* Pers., and *T. latifolia* L.) share extensive areas of sympatry (Figure 1), are becoming increasingly problematic invaders in numerous regions worldwide (Govaerts, 2004; Hall, 2009; Xu *et al.*, 2013; Bansal *et al.*, 2019; Maldonado, 2019), and hybridise (Ciotir et al., 2017; Smith, 1967). *Typha angustifolia* and *T. latifolia* share an extensive hybrid swarm across parts of eastern North America involving the F1 *Typha* × *glauca* and its advanced-generation and introgressed hybrids (Grace and Harrison, 1986; Tangen *et*

al., 2022; Bhargav *et al.*, 2022; Aleman *et al.*, 2024), and *T. domingensis* is known to hybridise with both *T. angustifolia* and *T. latifolia* (Ciotir et al., 2017; Smith, 1967); however little is known about the extent of hybrid zones involving *T. domingensis*. We also included two closely related species (*T. laxmannii* Lepech. and *T. shuttleworthii* W. D. J. Koch & Sond.; Zhou et al., 2018) with more restricted distributions (Figure 1; Govaerts, 2004), and shown as compatible to hybridise with both *T. angustifolia* and *T. latifolia* (Mavrodiev and Kapitonova, 2015). We sequenced multiple genomes from these five cattail species collectively sampled across multiple continents to test whether and how selection influenced their genetic divergence, and to investigate whether historical interspecific admixture or contemporary introgressions have contributed to intraspecific genetic diversity. In doing so, we aimed to expose the forces driving speciation among species with widespread distributions, old divergence times, and high levels of intra- and interspecific gene flow.

Materials and Methods:

Data preparation

Sampling, DNA extraction, and sequencing

Samples were obtained from previous studies as per Aleman et al. (2024) and supplemented with additional collections to increase taxonomic diversity and sampling range (Figure 1; Supplementary Table S1). Samples were initially identified to putative taxon using a combination of 3-4 microsatellite loci (Ciotir et al., 2013, 2017; Ciotir & Freeland, 2016) or known morphological characteristics (Grace & Harrison, 1986; Smith, 1967). The DNA was extracted following published protocols (Ciotir et al., 2017; Pieper et al., 2017, 2020) and converted into Nextera XT libraries as per Aleman et al. (2024) (*Appendix S1 – Detailed Methods*). Paired-end sequencing was executed on a Miseq (151 bp) and a Novaseq 6000 (126 bp) at The Centre for Applied Genomics (Toronto, Ontario, Canada) for 64 *T. angustifolia*, 25 *T. domingensis*, 104 *T. latifolia*, 11 *T. laxmannii*, and 3 *T. shuttleworthii*.

Raw data processing

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 pruning were carried out with trimmomatic 0.39 (Bolger *et al.*, 2014), removing cleaned reads shorter than 100 bp. All reads were mapped to the *T. latifolia* nuclear (15 chromosomes, 285.11 Mb,

GenBank accession no. JAIOKV000000000.2; Widanagama et al. (2022)) and chloroplast (161.57 kb, GenBank accession no. NC_013823; Guisinger et al. (2010)) genomes using the mem module of BWA 0.7.17 (Li and Durbin, 2009). Mapping statistics were evaluated with the flagstat and coverage modules of SAMtools 1.15.1 (Li *et al.*, 2009).

Genotype-calling

Genotype-calling was performed with ANGSD 0.93 (Korneliussen *et al.*, 2014) following the SAMtools model. Two datasets were created using all samples. First (*SNPs dataset*), SNPs were retrieved establishing minimum mapping and sequencing qualities = 20 (*-doGeno 4 -gl 1 -skipTriallelic 1 -SNP_pval 1e-6 -minMapQ 20 -minQ 20 -doMajorMinor 1 -domaf 1 -doPost 1 -doBcf 1*). The second dataset (*sequence dataset*) included all SNPs and invariant sites and was produced using the same parameters minus the filter for SNPs (i.e., without *-SNP_pval 1e-6*). For the *SNPs dataset*, SNPs with more than 20% missing data across all samples and sites mapped to the plastome were removed in VCFtools 0.1.16 (Danecek *et al.*, 2011). The plastome data were also removed from the *sequence dataset*, and no further filters were applied to this dataset. No individuals were filtered out of any of both datasets. The absence of clones (multiple ramets from the same genet) was verified by calculating kinship coefficients in Plink 2.0 (Chang *et al.*, 2015) with the *SNPs dataset*.

Genetic structure

We used the *SNPs dataset* to assess the most likely number of genetic clusters across all samples and the membership of each sample to these clusters, with three complementary approaches. ADMIXTURE 1.3.0 (Alexander & Lange, 2011) was run exploring $K = 1 – 10$. ADMIXTURE is known to fit groups that contain fewer samples as mixes of multiple groups rather than assigned to their own cluster (Lawson *et al.*, 2018); hence, due to the small sample size of *T. shuttleworthii* (n = 3) and following Singh et al., (2019), the most likely value of *K* was chosen for its congruence with the PCA plots (PC1 vs PC2 and PC1 vs PC3; Figure 2) and a neighbour-joining (NJ) tree (Figure 2) based on the samples' pairwise nuclear genetic distances (expressed as allele counts, transformed on the R 4.2.1 package ape 5.7-1 (Paradis and Schliep, 2019; R Core Team, 2022) generated with Plink 1.9 (Purcell *et al.*, 2007).

Genomic landscapes of speciation

To measure interspecific genetic divergence, F_{ST} (Weir and Cockerham, 1984), d_{XY} (Nei and Miller, 1990) and π (Nei and Li, 1979) were computed in 1 kb windows for all species pairs with pixy 1.2.7 (Korunes and Samuk, 2021) using the *sequence dataset*. The means were calculated, and d_a (d_{XY} – mean π), defined as the number of net nucleotide substitutions per site between species (Nei, 1987), was computed as a

measure of the relative distance between species along the speciation continuum.

We identified islands and valleys of divergence consistent with alternative types of selection between all species pairs. Following Kessler et al. (2023) and Shang et al. (2023), windows (1 kb) of exceptional genetic divergence (highly increased or reduced) were identified by detecting outliers for F_{ST}, d_{XY}, and π between species (higher or lower than the 95% quantile) plus intermediate values of d_{XY} (within the 45% and 55% quantiles). Islands and valleys of divergence were classified according to the four types of selection exposed by Irwin et al. (2018): i) divergence with gene flow (high F_{ST} and d_{XY}, but low π), ii) divergence without gene flow (high F_{ST}, intermediate d_{XY}, and low π), iii) background selection (high F_{ST}, low d_{XY} and π), and iv) balancing selection (low F_{ST}, and high d_{XY} and π). As neutrality was our null hypothesis, any window that could not be classified according to one of these types of selection was considered evidence for divergence as a result of genetic drift. BEDTools intersect (Quinlan and Hall, 2010) was used to extract any gene within windows driven by selection from the *T. latifolia* functional annotation (Widanagama *et al.*, 2022).

Historical admixture events and contemporary genetic introgressions

We inferred past interspecific hybridisation events (post-speciation) with the *SNPs dataset* in TreeMix 1.13 (Pickrell and Pritchard, 2012). We

performed linkage pruning (1 kb), and the analyses were run testing 0 - 10 migration events, applying the default bootstrap. The best number of migrations was chosen according to the total explained variance.

Using the *sequence dataset*, we looked for contemporary genetic introgressions between multiple pairs of species by making combinations of P1, P2, and P3 based on the species relationships inferred by TreeMix (Figure 1) and establishing *T. laxmannii* as the outgroup (Supplementary Table S2). The f_d (Martin et al., 2015), an estimator of the excess of alleles shared between two non-sister species that cannot be explained by incomplete lineage sorting, was computed using the script ABBABABAwindows.py (Martin & Jiggins, 2017) in 1 kb windows. Windows with a positive f_d plus an F_{ST} lower than the 5% quantile were classified as potentially introgressed, and introgressions were confirmed through visual inspection as in Rendón-Anaya et al. (2019). BEDTools intersect was used to extract the gene names across the introgressed blocks from the *T. latifolia* functional annotation.

Results

Genetic structure and evolutionary relationships

The final data set consisted of 89,726 SNPs with mean missing data per sample = 17.3%. The genetic structure analyses indicated the most likely number of species as five (Figure 2). The PCA showed some overlap along PC1 among *T. angustifolia*, *T. domingensis*, and T. *laxmannii*, as well as PC2 between *T. latifolia* and *T. shuttleworthii*, and between *T. domingensis* and T. *laxmannii*; while PC3 distinguished *T. domingensis* from *T. laxmannii*, explaining almost the same variance as PC2 (15.8% and 17%, respectively). In ADMIXTURE, two distinct intraspecific lineages for *T. latifolia* were detected, broadly identified as "Western" (North America, Western and Central Europe) and "Eastern" (Eastern Europe and the Iturup Island, in the Russian Kuril chain); *T. angustifolia* was also split into two lineages by ADMIXTURE, with samples from North America and Eastern Europe each assigned to a different cluster, plus a group of admixed individuals from Western Europe. Thus, *K* was set to seven (*K* = 7) based on visual inspection of the PCA plots and the two lineages within each of *T. latifolia* and *T. angustifolia*. The NJ tree differentiated all species and the intraspecific lineages of *T. latifolia* and *T. angustifolia*.

Genomic landscapes of speciation

Covering ~40% of the nuclear genome with 2× depth per sample, the mean π varied from 0.0051 (*T. latifolia*) to 0.0232 (*T. domingensis*), and d_a

ranged from 0.0074 (*T. latifolia* - *T. shuttleworthii*) to 0.0144 (*T. latifolia* - *T. angustifolia*) (Figure 2). The mean paired F_{ST} varied from 0.30 (*T. angustifolia* - *T. domingensis*) to 0.61 (*T. latifolia* - *T. laxmannii*), and d_{XY} ranged from 0.016 (*T. latifolia* - *T. shuttleworthii*) to 0.03 (*T. domingensis* - *T. laxmannii*) (Table 1). The islands and valleys of divergence were predominantly neutral; only 0.75% of the scanned genomic windows could be associated with one of the four types of selection. Most genomic regions related to selection were valleys of balancing selection, followed by islands of background selection (Table 2; Supplementary Table S3).

Historical admixture events and contemporary genetic introgressions

TreeMix indicated *T. angustifolia* - *T. domingensis* and *T. latifolia* - *T. shuttleworthii* as sister lineages and *T. laxmannii* as the outgroup species (Figure 1). The most likely number of migrations (historical admixture events) was zero, explaining over 99.9% of the total data variance, suggesting no evidence of historical gene flow.

The f_d identified contemporary putative introgressions in three genomic blocks on chromosomes 11 (~100 kb) and 14 (~70 and ~250 kb) from *T. latifolia* to both *T. angustifolia* and *T. domingensis*. Seven genes are encoded in the ~250kb (chromosome 14) block: *UBC16*, *TRX1*, *RTE1-homolog*, *EBS*, *WDR13*, *PAP2*, *and RGP1*. No other genes were identified in the ~100 kb (chromosome 11) and ~70 kb (chromosome 14) blocks. Additional potential introgressions between *T. domingensis* - *T. latifolia* and

T. angustifolia - *T. shuttleworthii* were found; however, the genomic landscapes revealed this was caused by balancing selection favouring polymorphisms between non-sister species, hence leading to an excess of shared alleles that could not be attributed to incomplete lineage sorting (see Smith & Hahn, 2023).

Discussion

During allopatric speciation, genetic divergence accumulates as a by-product of physical isolation and the evolutionary forces that each population experiences (Abbott *et al.*, 2013). By contrast, speciation in sympatry or parapatry requires a genetic basis (Rundle and Nosil, 2005). Ecological speciation can lead to stronger interspecific reproductive barriers than allopatry (Rundle and Nosil, 2005; Stukenbrock, 2014). Thus, speciation of currently hybridising species is predicted to be a consequence of episodes of allopatry and secondary contact, during which divergence accumulates, and gene flow gradually declines (Rheindt and Edwards, 2011; Sobel, 2016). We examined the genomic landscapes of five widespread, sympatric, and hybridising *Typha* species to identify whether and how selection influenced their genetic divergence and test whether past admixture events or contemporary introgressions have occurred among them. We observed predominantly neutral islands and valleys of divergence, and we detected contemporary introgressions in three species but no evidence of historical hybridisation. Our results coincide with patterns expected under allopatric speciation and genetic divergence driven primarily by drift, followed by species range expansion and secondary contact. This is important for the conservation of wetlands; the historical processes identified here are key to explaining the widespread invasive hybrid *T.* × *glauca* (*T. angustifolia* × *T. latifolia*) in Eastern North America and also

reiterate to the important role of human-mediated species range expansions and dispersal in novel invasive hybrids (Ellstrand and Schierenbeck, 2000; Mesgaran *et al.*, 2016).

Absence of adaptive divergence in Typha

Investigating how species originate is crucial to understanding the processes that produce and preserve biological diversity (Hernández-Hernández *et al.*, 2021). The modes of speciation can have lasting effects on processes such as ongoing hybridisation and, thus, on the potential origin of hybrid biological invaders (Stukenbrock, 2014). To evaluate if speciation in *Typha* resulted from divergent selection, we looked at islands and valleys of divergence between pairs of *Typha* species. The genomic landscapes of speciation in our study were primarily related to neutral divergence: less than 1% of the genome indicated selection as the driver of divergence, which suggests that speciation occurred in allopatry and was primarily driven by drift. Alternatively, if natural selection was the driver of speciation, the signatures could have been eroded through time and recombination –which is consistent with late stages of speciation (Burri *et al.*, 2015), i.e., species can become so divergent that the recombination suppression is re-established regarding any barrier loci (Schluter and Rieseberg, 2022). While the F_{ST} values could be interpreted as far along the speciation continuum (0.30 - 0.61), the d_a estimates are within the 'grey zone of speciation' (d_a between 0.005 and 0.02; Roux et al., 2016),

indicating that divergence and reproductive isolation among these *Typha* is weak, as confirmed by the hybridisation between multiple pairs species (Smith, 1967). This current lack of reproductive barriers between multiple *Typha* species (Smith, 1967) and the absence of historical gene flow detected by Treemix are consistent with the species in our study being historically allopatric and only more recently sympatric.

Balancing selection and introgressions as sources of genetic variation

Standing genetic variation can be as important to adaptation as *de novo* mutations (Barrett and Schluter, 2008; Matuszewski *et al.*, 2015). Several mechanisms can maintain standing genetic variation, including balancing selection (Llaurens *et al.*, 2017). Across the genomic landscapes of divergence among the *Typha* spp. in our study, most regions associated with adaptive divergence were valleys of balancing selection, in which the maintenance of ancestral polymorphisms causes an excessively reduced divergence. This means natural selection favours heterozygosity at some loci regardless of the species (Guerrero and Hahn, 2017). In plants, genes under balancing selection influence ecological distributions, promote adaptation to divergent habitats (Wu et al., 2017), and underlie a wide range of phenotypes (Isildak *et al.*, 2021). Balancing selection has similarly influenced genetic variation in several widespread tree species, including *Arbutus*, *Populus* and *Quercus* (Santiso *et al.*, 2016; Meireles *et al.*, 2017; Wang *et al.*, 2020; Rendón-Anaya *et al.*, 2021; Shang *et al.*, 2023). Our

results highlight the role of balancing selection in preserving diversity at ecologically relevant traits among plant species despite the accumulation of genetic divergence (Delph and Kelly, 2014; Selechnik *et al.*, 2019).

In addition to balancing selection, introgressions are potentially important sources of standing genetic variation (Tigano and Friesen, 2016; Suarez-Gonzalez *et al.*, 2018). Research across the *T.* × *glauca* hybrid swarm (the hybrid of *T. angustifolia* × *T. latifolia*) has exposed the sexual fertility of F1 hybrids (Snow *et al.*, 2010), a high frequency of F1 hybrids in natural populations (Travis *et al.*, 2010; Kirk *et al.*, 2011), and the capability of F1 hybrids to backcross (Bhargav et al., 2022; Pieper et al., 2017). Under these circumstances, introgressions between *T. latifolia* and *T. angustifolia* were expected. Three genetic blocks were identified as introgressed between *T. latifolia* and *T. angustifolia*, one of which was associated with seven genesl; the genes encoded in the ~250 kb block contribute to cellular metabolism, regulation, and response mechanisms to oxidative stress. The introgressed blocks were also detected between *T. latifolia* and *T. domingensis*, for which hybridisation was identified by Smith (1967) and Ciotir et al. (2017). Introgressions have been documented in numerous other widespread plant species, including *Helianthus* (Whitney *et al.*, 2006, 2010), *Populus* (Rendón-Anaya *et al.*, 2021; Shang *et al.*, 2023) and *Quercus* (Leroy *et al.*, 2020), and are recognised as essential sources of genetic variation. Introgressions in *T. angustifolia*, *T. latifolia*, and *T.*

domingensis are congruent with the idea that hybridisation may be more likely when speciation occurs in allopatry followed by secondary contact (Levin, 2012; Harrison and Larson, 2014).

Genetic diversity in Typha and T. latifolia

Genetic diversity reflects the reservoir of traits and potential responses to environmental changes that species encounter, thereby influencing their success and resilience in dynamic ecosystems (Gregorius, 1987; Frankham *et al*., 2002; Hartl and Clark, 2006). Excluding *T. latifolia*, intraspecific genetic diversity was high ($\pi > 0.01$; see Begun et al., 2007), which is congruent with *Typha* spp. large (effective) population sizes and substantial gene flow. In *T. latifolia*, genetic diversity was substantially lower than any species in this study despite having the broadest geographic distribution; this was consistent with Mashburn et al. (1978), Keane et al. (1999), and Pieper et al. (2020), who detected extremely low genetic diversity in *T. latifolia* in North America. Low genetic diversity is considered detrimental since it can reflect a low adaptive potential (Frankham *et al.*, 2017). However, the some species – including biological invaders – thrive despite a low genetic diversity (Tsutsui *et al.*, 2000; Roman and Darling, 2007). *Typha* is globally distributed and tolerates a broad range of climates, nutrients, pH, and water level fluctuations (Kadlec & Wallace, 2008; Sojda & Solberg, 1993), and *T. latifolia* is the most widespread and commonly recognised *Typha* spp., presumed to have large

census sizes, and is considered invasive in Oceania (Xu *et al*., 2013). Reduced genetic diversity in *T. latifolia* suggests that alternative mechanisms like epigenetic modifications and associated phenotypic plasticity could be responsible for the success of this species (Mounger *et al*., 2021). In *Arabidopsis thaliana*, epigenetic variation has been shown to underlie morphological variation and phenotypic plasticity when genetic diversity is low (Zhang *et al*., 2013; Kooke *et al*., 2015). A possible inquiry for future research is testing if epigenetic modifications are more extensive in *T. latifolia* than in other *Typha* species.

Conclusions

Determining the drivers of speciation is a central aspect of evolutionary biology. Here, we tested the role of selection and hybridisation in promoting species divergence in *Typha*, an ancient and widespread plant genus characterised by high levels of intra- and interspecific gene flow. The absence of barriers to hybridisation among some species in our study suggested that their speciation occurred in allopatry. Our results supported allopatry as the leading cause of speciation and the presence of balancing selection and introgressions in the evolutionary history of *Typha*. The absence of adaptive divergence does not rule out the role of selection as a promoter of speciation among *Typha* species in our study –the genic view of speciation requires only a handful of genes to drive speciation (Lexer and Widmer, 2008)–; rather, it indicates a primary role of genetic drift in

allopatry on this process. Likewise, this does not mean that *Typha* species are not adapted in any way to their local environment, and investigating local adaptation remains an essential research gap in this widespread plant genus. Relating the timing of speciation to decreased historical population sizes in *Typha* spp. could also be a future step to understanding the history of this genus, as our results suggest that the species in our study experienced periods of isolation despite their current widespread distributions. Finally, assessing the weight of drift versus selection in promoting speciation in a broader range of widespread and old species will allow us to comprehend how the evolutionary forces drive species divergence during speciation, and the roles of hybridisation and introgressions as sources of genetic variation.

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Author contribution statement

Conceptualisation, developing methods, conducting the research, data interpretation and writing: Aaron B. A. Shafer, Alberto Aleman, Joanna R. Freeland, and Marcel E. Dorken. *Data analysis and preparation of figures & tables*: Alberto Aleman.

All authors contributed to the manuscript and approved its final version.

Conflict of Interest

The authors declare no conflict of interest.

Data archiving

Data and scripts for this study can be found at https://gitlab.com/WiDGeT_TrentU/graduate_theses/-/tree/master/aleman/hdy and https://github.com/al-aleman/totoras_hdy. High throughput sequencing raw data are available from the authors upon reasonable request.

Research Ethics Statement

The reported research did not require any ethical permissions.

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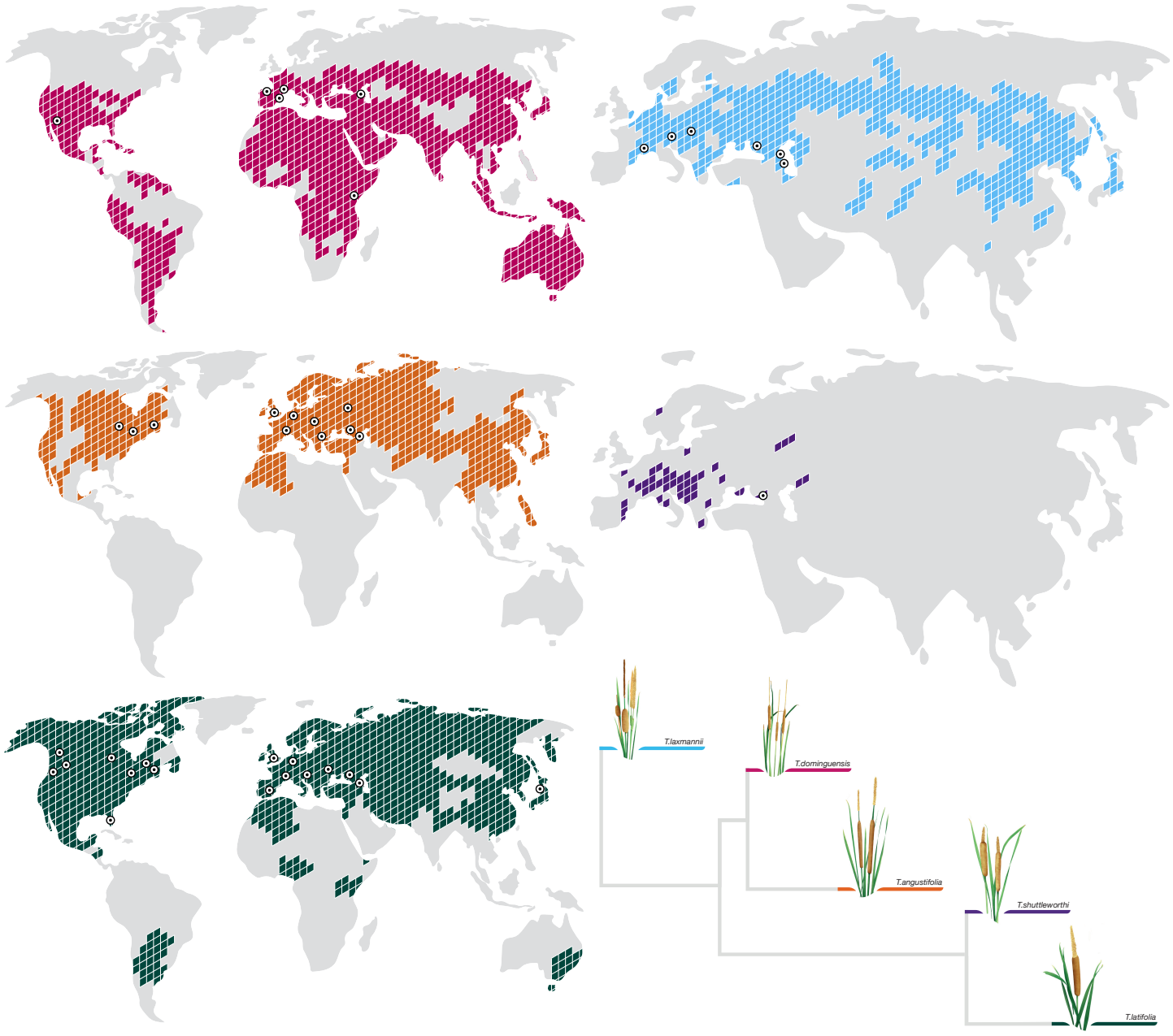
TABLES AND FIGURES

Figure 1. Contemporary distributions (coloured grids), approximate sample sites (black and white circles), and species relationships inferred by TreeMix in this study for *T. angustifolia* (top-left), *T. domingensis* (middle-left), *T. latifolia* (bottom-left), *T. laxmannii* (top-right), and *T. shuttleworthii* (middle-right). Distribution information from Ciotir & Freeland (2016) and GBIF. The number of samples collected is not shown. The maximum likelihood tree produced by TreeMix (bottom-right) indicated no historical hybridisation events, and branch lengths are not represented. Coloured areas represent different taxa, as in the tree.

Figure 2. Nuclear genetic structure and species relationships for five *Typha* spp. Top: Principal component analysis (PCA) for PC1 and PC2 (left) and PC1 and PC3 (right). Middle-left: Nucleotide diversity (π, inside shapes) for each species and relative distance (d_a, between shapes) between species. Shapes represent individuals, and colours represent taxa. Middle right: Neighbour-joining tree. Branches represent individuals, and colours indicate species and lineages. Bottom: ADMIXTURE plot for $K = 7$. Vertical bars represent individuals, and the admixture proportion is shown in different colours. A = *T. angustifolia* (“Western”), B = *T. angustifolia* (“admixed”), C = *T. angustifolia* (“Eastern”), D = *T. domingensis*, E = *T. latifolia* (“Western”), F = *T. latifolia* (“Eastern”), G = *T. laxmannii*, H = *T. shuttleworthii*.


T.laxmannii
T.dominguensis
T.angustifolia
T.shuttleworthii
T.latifolia

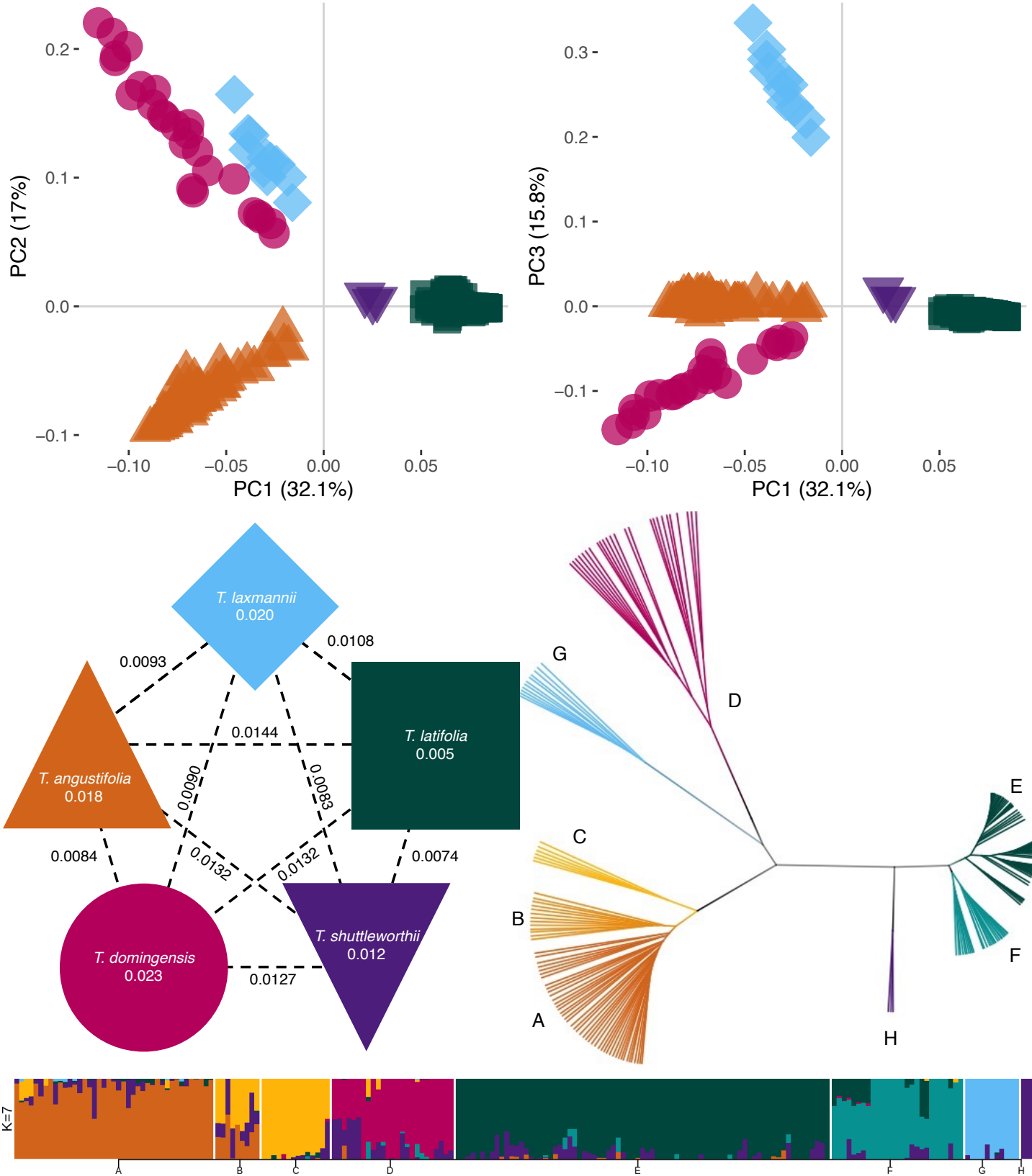

PC2 (17%)
PC1 (32.1%)
PC3 (15.8%)
PC1 (32.1%)
T. laxmannii
0.020
T. latifolia
0.005
T. angustifolia
0.018
T. domingensis
0.023
T. shuttleworthii
0.012
0.0093
0.0108
0.0144
0.0090
0.0083
0.0084
0.0132
0.0132
0.0074
0.0127
A
B
C
D
E
F
G
H
K=7

Table 1. Mean relative (F_{ST}, below diagonal) and absolute (d_{XY}, above diagonal) genetic divergence between five *Typha* species, measured in 1 kb windows.

	T. laxmannii	*T. domingensis*	*T. angustifolia*	*T. shuttleworthii*	*T. latifolia*
T. laxmannii		0.031	0.028	0.024	0.023
T. domingensis	0.321		0.029	0.030	0.027
T. angustifolia	0.390	0.304		0.028	0.026
T. shuttleworthii	0.377	0.381	0.449		0.016
T. latifolia	0.610	0.573	0.557	0.534	

Table 2. Number of genetic islands and valleys of divergence consistent with alternative types of natural selection among five *Typha* spp. DwGF = divergence with gene flow (in sympatry). AS = Divergence without gene flow (in allopatry). BGS = Background selection. BLS = Balancing selection. AllWin = Total number of windows (1 kb) scanned.

	DwGF	**AS**	**BGS**	**BLS**	**AllWin**
T. angustifolia - *T. domingensis*	0	73	188	807	211,136
T. angustifolia - *T. latifolia*	1	26	47	36	224,754
T. angustifolia - *T. laxmannii*	0	82	300	1,527	172,082
T. angustifolia - *T. shuttleworthii*	4	120	368	1,487	188,222
T. domingensis - *T. latifolia*	24	103	46	70	220,991
T. domingensis - *T. laxmannii*	0	39	348	1,497	171,478
T. domingensis - *T. shuttleworthii*	0	84	337	1,789	187,320
T. latifolia - *T. laxmannii*	50	186	73	304	175,533
T. latifolia - *T. shuttleworthii*	88	202	39	113	195,110
T. laxmannii - *T. shuttleworthii*	3	93	269	1,940	159,729