










RESEARCH ARTICLE

Climate and genome size shape the intraspecific variation in ecological adaptive strategies of a cosmopolitan grass species

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Abstract

1. Intraspecific trait variation, essential to Darwin's mechanism of natural selection, has been widely examined for single characters. However, intraspecific variation of adaptive strategies which represent trade-offs among multiple functional traits has received less attention, particularly for species that are globally distributed or invasive. Grime's competitor, stress tolerator and ruderal (CSR) scheme, well validated in the context of alien species invasions, provides both the theory and quantitative methodology to investigate adaptive strategies.
2. Here, we quantified the intraspecific CSR strategy scores of a worldwide collection of 89 *Phragmites australis* genotypes which were cultivated in a common garden. We assessed the relationships between intraspecific variation in CSR strategy scores, genome size and climate of origin and tested whether the invasive and native lineages differ in CSR strategies.
3. Substantial variation in intraspecific adaptive strategy, characterized mainly in C- and S-selection, was observed. As expected, C-, S- and R-scores showed clear latitudinal clines and were strongly related to either genome size or climate of origin. Furthermore, invasive lineages were more stress adapted than native lineages.
4. We conclude that the adaptive strategy of this perennial wetland grass varies globally, and its genotypes are surprisingly more stress tolerant in the invaded range where it was first detected ~150 years ago.

KEYWORDS

adaptive evolution, biological invasion, Grime's adaptive strategy, latitudinal cline, *Phragmites australis*

1 | INTRODUCTION

Intraspecific trait variation is of profound importance for species encountering environmental gradients, particularly for those with a large range size, exposed to dramatic environmental changes or introduced to novel habitats in the non-native range (i.e. alien species; Cronin et al., 2015; des Roches et al., 2017; Eller et al., 2017; Meyerson et al., 2024; Meyerson, Cronin, & Pyšek, 2016; Westerband et al., 2021; Yang et al., 2020). In fact, the idea that environmental differences may cause morphological differences within a species can be traced back to the fourth century BC when Theophrastus stated that 'all those trees which are common to both hill and plain are taller and finer in appearance when they grow in the plain' (Stegmann, 2021). Intraspecific differences in traits are increasingly recognized as a key component in determining individual and population performance, biotic interactions, community assembly and ecosystem properties (Cronin et al., 2015; des Roches et al., 2017; Violle et al., 2012; Westerband et al., 2021). They are also a fundamental prerequisite for Darwin's mechanism of natural selection and inevitably constitute a link between natural selection of individuals and the survival of individuals in communities. For instance, latitudinal clines in trait variation have been observed for *Hypericum perforatum* (Maron et al., 2004), *Impatiens glandulifera* (Helsen et al., 2020), *Phragmites australis* (Clevering et al., 2001; Cronin et al., 2015; Ren et al., 2020) and *Spartina alterniflora* (Liu et al., 2016). Although single or defined suite of morphological and physiological traits are often selected to study intraspecific variation, it is well known that functional traits are multidimensional and inter-related (He et al., 2020), and that the magnitude of variation among traits differs (Roybal & Butterfield, 2019). To better understand the forces underlying trait covariation across species, integration of multiple intraspecific traits, that is, trade-offs between traits, has recently been called for (Siefert et al., 2015; Vasseur et al., 2018; Violle et al., 2012).

Grime's competitor (C), stress tolerator (S) and ruderal (R), that is, CSR, theory is one scheme that facilitates the integration of trade-offs between traits (Grime, 1974; Grime & Pierce, 2012; Pierce et al., 2013, 2017). CSR theory assigns species a position relative to three primary (extreme) strategies based on the species' overall fitness to two limiting environmental factors: stress (i.e. metabolic limitation through resource limitation or cell component damage, constraining maximum growth rates) and disturbance (i.e. removal of plant parts or the whole organism, such as by grazing). Adapted to highly productive and low-disturbance habitats, competitive plants usually grow fast and large to outcompete neighbours; in habitats highly stressed by low resource availability, stress adaptors grow slowly and tend to invest limited resources in defence and protection (Grime & Pierce, 2012). Ruderals are adapted to highly productive but also to intensively or periodically disturbed habitats and can thus grow quickly and invest more resources in reproduction. Even though Grime proposed this adaptive strategy theory in the late 1970s, its application remained limited to certain regions, such as Great Britain (Grime, 1974, 1977; Hodgson et al., 1999) or the Czech

Republic (Pyšek et al., 2003). Recently, Pierce et al. (2017) provided a method for quantifying C-, S- and R-scores based on the combinations of key functional traits and further validated with the world flora. The proposed approach offers a valuable integration of Grime's CSR scheme with the widely recognized worldwide leaf economics spectrum, which explores the trade-off between resource capture and investment traits (Onoda et al., 2017; Wright et al., 2004), and the plant size gradient (Díaz et al., 2016; Pierce et al., 2022). This integration results in a comprehensive and unified global spectrum that effectively characterizes the diversity of plant form and function across different ecological contexts, also supported by a theory capable of explaining trait variation. By harmonizing these influential concepts, Pierce et al. (2017) provide a more holistic understanding of the relationships between key ecological traits, fostering a deeper comprehension of plant ecology on a global scale and facilitating the application of the scheme and the assessment of species CSR adaptive strategies. For instance, Guo, van Kleunen, et al. (2018) used CSR scores to test adaptation strategies driving alien plant naturalization and found that C- or R-selected species are more likely to become naturalized compared to S-selected species.

Although initially proposed for species-level studies, the adaptive strategy scheme has also been used to investigate the intraspecific variation in certain species, such as *Arabidopsis thaliana* (May et al., 2017; Vasseur et al., 2018), the model species in molecular biology and quantitative genetics; *Campanula elatinoidea*, endemic to the Lombardy pre-Alps (Giupponi, 2020); *Silene paradoxa* growing on serpentine and non-serpentine sites (Lazzaro et al., 2020); and multiple native and non-native species along an elevational gradient (Hasigerili et al., 2023). All of these studies found substantial variation in adaptive strategies (i.e. varying CSR scores) within the examined species and attributed the observed variation in adaptive strategy to intraspecific variation in species' adaptation to local environment (Giupponi, 2020; Hasigerili et al., 2023; May et al., 2017; Vasseur et al., 2018). Lazzaro et al. (2020) further found a shift in the gene pool in *S. paradoxa* grown on serpentine and non-serpentine soils, providing convincing evidence of species' adaptation to different soil conditions. Vasseur et al. (2018) applied $Q_{ST} - F_{ST}$ analysis to investigate the role of adaptive or non-adaptive processes in strategies' variation and found that the adaptive strategy in *A. thaliana* is likely due to natural selection of leaf traits. These studies have proven that intraspecific variations in CSR scores are common in plant species, most likely resulting from local adaptations. However, these studies were either restricted to relatively small spatial regions or used cultivated species. Thus, results from a wild species with a worldwide distribution could provide more robust conclusions with regard to intraspecific variation in Grime's strategies and the underlying associations, benefiting from the extensive climatic variations in which such species occur. Such an approach could thus provide insights into how plant adaptive strategies could evolve in response to future climate changes (Eller et al., 2017).

Recently, *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae) has been proposed as a model species for the study of ecophysiological

responses to environmental gradients (Eller et al., 2017), biological invasions (Meyerson, Cronin, & Pyšek, 2016; Packer et al., 2017) and plant science more generally (Cesarino et al., 2020), due to its extensive distribution worldwide, substantial phylogeographic genetic diversity (Lambertini et al., 2006, 2012, 2020; Saltonstall, 2002) and large variation in functional traits (Ren et al., 2020). *Phragmites australis* is one of the world's most cosmopolitan wetland grasses, distributed on all continents except for Antarctica (Guo et al., 2013; Packer et al., 2017). It is a helophytic perennial grass with a maximum height record of 5 m and can reproduce thousands of seeds per inflorescence (Mckee & Richards, 1996; Packer et al., 2017). Vegetative reproduction is ensured by the extensive production of rhizomes and stolons (Packer et al., 2017; Pyšek et al., 2019). Many of the ecophysiological traits of *P. australis* populations collected worldwide show significant latitudinal patterns, even when grown in different common gardens several thousand kilometres apart, indicating clinal adaptation of those traits at their collection sites (Bhattarai et al., 2017; Haslam, 1975; Meyerson et al., 2020; Ren et al., 2020). In addition, it also exhibits extensive phenotypic plasticity in response to environmental changes (Clevering et al., 2001; Eller et al., 2014; Meyerson et al., 2020; Mozdzer et al., 2016), particularly the invasive haplotype M lineage, which originated from Eurasia and introduced to North America approximately 150 years ago (Allen et al., 2020; Saltonstall, 2002). It has been demonstrated that this invasive lineage was likely preadapted to become invasive, but also became more invasive as it evolved and adapted in the introduced range (Guo et al., 2014; Guo, Lambertini, et al., 2018).

The evolution of advantageous traits in the new range could be associated with the small genome size of the invasive populations (Meyerson et al., 2024). According to the 'large genome constraint' hypothesis (Knight et al., 2005; Meyerson et al., 2020; Suda et al., 2015), individuals with smaller genomes can survive, reproduce and disperse more effectively due to traits such as short generation times, fast growth rates and small seed size (Grotkopp et al., 2004; Knight et al., 2005; Suda et al., 2015). Even though the hypothesis was proposed, and is usually applied, at the species level (e.g. Guo et al., 2024; Pyšek et al., 2023), recent investigation within populations has also provided validation. For instance, Pyšek et al. (2018) demonstrated that out of 15 traits representing karyology, growth, reproduction, physiology, herbivory and tissue chemistry, monoploid genome size (the amount of DNA in one chromosome set, sensu Greilhuber et al., 2005) was the only important trait differentiating North American native *P. australis* populations from the invasive haplotype M. Invasive populations with a smaller genome size were also characterized by relatively extensive rhizomes, earlier shoot emergence, greater resistance to aphid attack and lower C:N ratio than the native populations. Similarly, *Centaurea solstitialis* populations with smaller genome size had higher growth rates and above-ground biomass and reproduced earlier than individuals with larger genome sizes (Cang et al., 2024). Recently, relationships between genome size and adaptive strategies have been observed in large multispecies

studies (Guignard et al., 2016; Guo et al., 2024). However, compared to an approximately 2400-fold variation in genome size among species (Pellicer et al., 2010; Pellicer & Leitch, 2020), it remains uncertain the degree to which the lesser variation of genome size across individuals and populations (intraspecific variation) is important to ecological adaptation.

Here, we examine the intraspecific CSR strategy scores of 89 *P. australis* genotypes collected worldwide and cultivated in a common garden in the Czech Republic (Pyšek et al., 2019). We measured the relevant functional traits and calculated each genotype's CSR score based on the methods of Pierce et al. (2017). Briefly, this CSR calculation method attributes C, S and R values to each individual plant or species based on the relative extent of leaf trait values (see below for trait and measurement details), which are compared statistically to trait variation in the world flora. We also used genome size as an important trait to reveal possible relationships between genome size and CSR strategies. We tested the following hypotheses: (1) climate of origin and genome size are associated with significant variation in CSR strategy scores, and (2) certain phylogeographic groups exhibit different CSR strategies. Specifically, the invasive lineage exhibits greater C-selection than the native North American lineage, and a significant shift in CSR scores can be detected between the invasive lineage in North America and its European source population due to post-introduction evolution.

2 | METHODS

2.1 | Population sampling

Phragmites australis genotypes used in the study represent distinct phylogeographic groups worldwide (Lambertini et al., 2006; Pyšek et al., 2019). Following Saltonstall (2002, 2016), chloroplast DNA was extracted to determine haplotypes and phylogeographic lineages. In total, our sample sets included 21 native European genotypes and 17 genotypes introduced to North America with haplotype M (hereafter Europe and Namerica_inv, respectively), 19 native North American genotypes (hereafter Namerica_nat, including multiple haplotypes), 17 from Australia (haplotype P), seven from the Far East (Sakhalin Island) (haplotypes P and O) and eight from South Africa (hereafter SAfrica, haplotype K) (detailed information concerning the collection locations of the genotypes are presented in Figure S1 and Table S1). The phylogenetic relationships among these lineages are well established (Lambertini et al., 2006, 2012, 2020), as well as the differences among these lineages maintained in common garden in ecophysiological and morphological traits, and biomass (Eller & Brix, 2012; Nguyen et al., 2013; Guo et al., 2014, 2016; Pyšek et al., 2019; Ren et al., 2020). The 89 genotypes were obtained from the collections at the common gardens at the University of Rhode Island (USA) and Aarhus University (Denmark), or directly from the wild (Pyšek et al., 2019). Except for the two genotypes collected from the wild

in the Czech Republic, all other genotypes were cultivated in the common gardens for more than 5 years (Lambertini et al., 2006, 2008; Meyerson et al., 2020; Ren et al., 2020). In all cases the genotypes were originally collected from distant populations as shown in Figure S1 and Table S1 and are not obviously related to each other. On the contrary, the genetic variation pattern of *P. australis* shows a predominantly long-distance seed-dispersal pattern all over the world (Canavan et al., 2018; Hurry et al., 2013; Lambertini et al., 2008, 2020; Saltonstall, 2003). This reduces spatial autocorrelation in the data due to genetic similarities among genotypes within phylogeographic groups.

2.2 | Experimental set-up

The experiment was carried out in the experimental garden of the Institute of Botany CAS in Průhonice, Czech Republic (49°59'38.972" N, 14°33'57.637" E), with an elevation of 320 m a.s.l., mean annual temperature and precipitation of 8.6°C and 610 mm (Pyšek et al., 2019), respectively. The rhizomes of the selected genotypes were propagated in sand culture in 2011. A length of 20–30 cm of rhizome of each genotype was planted in a round pot on 7–8 July 2012, with two to six replicates per genotype, depending on the availability of the plant material. The round pot was 60 cm in diameter at the top and 36 cm in height, filled with sand mixed with 480 g of slow-release fertilizer (Osmocote Pro; providing essential macro and micronutrient elements over a 12–14-month release period). In total, 273 pots were used in the experiment. The pots were tap watered regularly by an automatic watering system (Hunter Industries, San Marcos, USA), and a standard water level in pots was achieved by three holes in the pot 25 cm from the bottom that drained excessive water from the pot. Plants were checked frequently, and iron deficiency (leaf yellowing) was cured with a solution of a chelation complex of DTPA and iron (consisting of 0.2 g Fe dissolved in 300 mL tap water that was added to each pot). In October of each year of the experiment, above-ground biomass was harvested, and pots were covered with spruce brushwood and bubble foil to provide isolation from potential frost damage. The cover was removed in early April and 200 g of Osmocote and 0.2 g Fe was added to each pot, with an addition of the same Fe dose added in May/June.

2.3 | Relevant traits measured

In August of 2014, we measured the three leaf traits used to calculate the CSR scores: leaf area (mm², LA), specific leaf area (the ratio of leaf area to leaf dry mass, mm² g⁻¹, SLA) and leaf dry mass content (g dry mass g⁻¹ fresh mass, LDMC) (for a complete list of traits measured, see Pyšek et al., 2018, and the appendix S2 and table S1 therein). Specifically, the third or fourth fully developed top leaf in four randomly selected shoots per pot were measured for LA

using LICOR Area Meter (LI-3100, LI-COR, Lincoln, Nebraska, USA). Those leaves were weighed individually to obtain the fresh mass and then oven-dried at 60°C for 48 h at least for dry mass. SLA was calculated as LA/dry mass, and LDMC was calculated as leaf dry mass/fresh mass. These three traits were used to calculate the CSR scores for each genotype via the globally calibrated CSR analysis tool 'StrateFy' (Pierce et al., 2017). Note that although CSR scores are calculated from these particular traits, they are among the most important descriptors of leaf resource economics and size (the principle functional spectra of plants; Díaz et al., 2016) and are correlated with a range of additional whole plant, organ and seed traits, including traits such as leaf nitrogen content, plant height and seed mass (Pierce et al., 2017). Also, the C, S and R scores do not directly represent single trait values, but the relative investment in opposed traits and thus the trade-offs between them (sensu Grime & Pierce, 2012), thereby representing the resulting C-R-S adaptive strategy of each species or individual (Pierce et al., 2017).

Monoploid genome sizes, that is, the amount of DNA in one chromosome set with chromosome number *x* (Cx-value), were calculated as 2C-value/ploidy level. The genome size (2C-value) of *P. australis* genotypes was determined by DNA flow cytometry by a simplified two-step protocol (see Doležel et al., 2007; Sliwinska et al., 2022) using Otto buffers. Briefly, ~0.5 cm² of fully developed intact leaf tissue was chopped with an appropriate amount of internal standard *Bellis perennis* (2C = 3.38 pg; Schönswetter et al., 2007) with a sharp razor blade in a Petri dish containing 1 mL of Otto Buffer I (0.1 mol/L citric acid, 0.5% Tween 20). The suspension was filtered through a 42 µm nylon mesh and incubated for 20 min at room temperature. Samples were stained for 10 min at room temperature with 1 mL of Otto Buffer II (0.4 mol/L Na₂HPO₄ · 12 H₂O) supplemented with β-mercaptoethanol (2 µL/mL) and intercalating fluorochrome propidium iodide + Rnase IIA (both at concentrations 50 µL/mL). The samples were run in a Sysmex-Partec CyFlow SL device equipped with a green (532 nm, 100 mW output power) solid-state laser. Flow cytometry measurements were run for 5000 particles and were repeated at least three times on each plant on different days to minimize potential random instrumental drift, and only a between-day variation of less than 2% was utilized for further analysis.

2.4 | Climatic variables

The 19 commonly used bioclimatic parameters in the WorldClim database (Hijmans et al., 2005) were extracted for each genotype to build a principal component analysis (PCA) summarizing the variations among the parameters and improve explanation power (Dupin et al., 2011). The first three PC axes explained ~85% of the variation of the 19 parameters. The first axis (PC1) mostly represented the gradient of mean annual temperature, the second axis (PC2) precipitation during wet or warm periods and the

third axis (PC3) precipitation during drought periods (Table S2). The three PCs were used as bioclimatic variables in the following analyses.

2.5 | Statistical analysis

The CSR scores of each genotype were presented in a ternary plot showing the global intraspecific variation of *P. australis*, using the *ggtern* package (Hamilton & Ferry, 2018) in the R 4.2.0 environment (R Core Team, 2022). The relationships between each of the C-, S- and R-scores and latitude were tested via linear regression. We then applied generalized linear mixed models for each of the C-, S- and R-scores to test the effects of monoploid genome size and the three bioclimatic PCs with the phylogenetic groups as the random factor via the *lme4* package (Bates et al., 2015). Multicollinearity was checked for each model using variance inflation factor (VIF) scores, and all VIFs were <4, indicating rather negligible collinearity between the variables (Dormann et al., 2013). To check whether the adaptive strategies of *P. australis* in general are influenced by the four predictors, we further ran a permutational multivariate analysis of variance via the *vegan* package (Oksanen et al., 2022). To test the differences of each of the C-, S- and R-scores among invasive and non-invasive lineages in North America, we ran the K-sample Fisher–Pitman permutation test among North American invasive, European native and North American native lineages with 100,000 permutations in the *coin* package (Hothorn et al., 2008). A permutational multivariate analysis of variance was also employed to test the differences of the adaptive strategies of *P. australis* among the three groups via the *pairwiseAdonis* package (Martinez, 2020).

3 | RESULTS

3.1 | Global intraspecific strategy variation of *Phragmites australis*

With mean C-, S- and R-scores of 37.7%, 60.8% and 1.5%, respectively, *P. australis* is generally a competitive stress-adapted (CS-selected) species. Crucially, a wide range of CSR scores occurred among *P. australis* genotypes, varying particularly along the C- to S-axis (Figure 1). Among the six phylogeographic groups, Australian genotypes showed the most extensive variations along the S-axis, whereas both South African and invasive North American populations were more tightly clustered even though the sampling locations of the latter were spread over a much wider geographic range than those of the Australian genotypes (Figure S1). Notably, the Far East genotypes also exhibited relatively extensive variation, although samples were all collected in the same geographic region.

The extent of C-, S- and R-selection exhibited highly variable but significant correlations with latitude (Figure 2 and Figure S2): the C- and S-scores, respectively, showed negative and positive relationships with latitude ($p < 0.05$) (with low R^2 values, the relationships

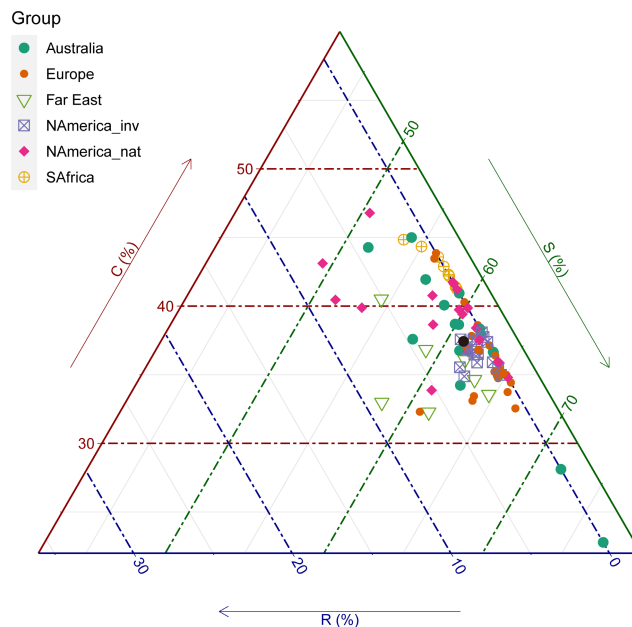


FIGURE 1 C-S-R classification of *Phragmites australis* genotypes collected globally. Symbols and colours indicate different phylogeographic groups (NAmerica_inv: North American invasive; NAmerica_nat: North American native; SAfrica: South Africa), and the black circle indicates the mean CSR-score value of *P. australis*. The C-S-R classification was determined based on the method of Pierce et al. (2017). Note the truncated axis ranges to allow clearer visualization.

were not strict enough that values of C- or S-selection can be accurately predicted from latitude, but the low p values indicate that changes in C- or S-selection are at least associated with changes in latitude). Although the maximum absolute R-score for our global samples was less than 8%, and scores for many genotypes were equal to zero (Figure 1; i.e. R-selection was less evident than C- and S-selection across the species), a significant, positive latitudinal pattern was also observed for the R-score ($p = 0.04$, Figure S2).

3.2 | Relationships between monoploid genome size, climate and global CSR variation

The global CSR variation of *P. australis* was strongly associated with both genome size and climate at origin, although their effects were different for each score ($p < 0.05$, Table 1, Figure 3 and Figure S3). The extent of C-selection was positively and negatively related to the variation in monoploid genome size and climate PC2 (precipitation during wet or warm periods, Figure 3a,b), while the S-score showed opposite patterns with regard to each of the two variables (Figure 3c,d). As for the C-score, the R-score was also positively associated with monoploid genome size; however, it showed a negative relationship with climate PC1 (mean annual temperature; $p < 0.05$, Figure S3). In addition, the multivariate analysis found that both genome size and the first two climate PCs were significantly associated with the intraspecific adaptive strategy variations of *P. australis* ($p < 0.01$, Table S3).

3.3 | Intraspecific strategy variation and invasion by the haplotype M lineage

The native European and invasive North American lineages exhibited similar C- and S-scores, which were either significantly lower or higher than the values of the native North American lineage

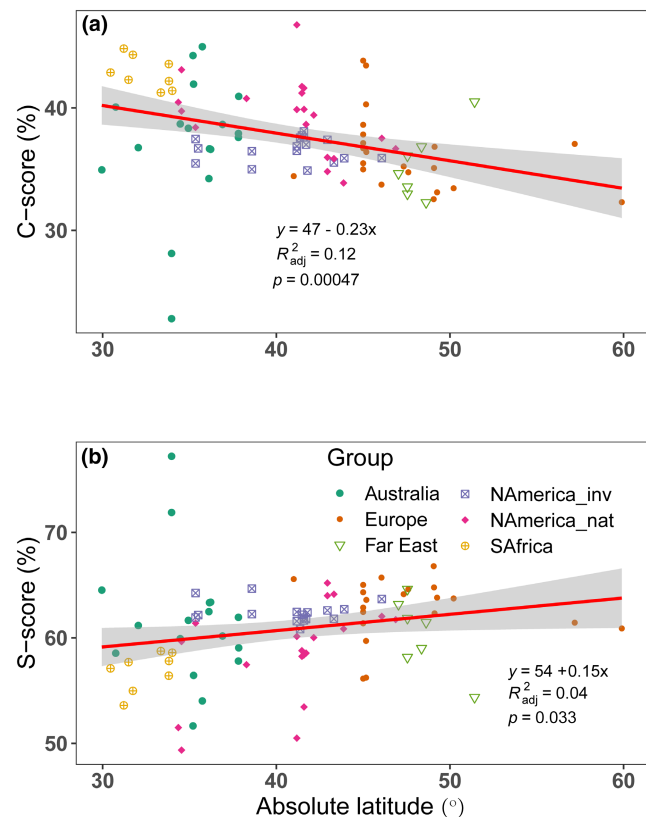


FIGURE 2 Latitudinal clines in (a) C- and (b) S-scores of *Phragmites australis*. Symbols and colours indicate different phylogeographic groups. NAmerica_inv, North American invasive; NAmerica_nat, North American native; SAfrica, South Africa.

TABLE 1 Summary of the generalized linear mixed models of C-score and S-score. The first climate PC (PC1) attributed mainly to mean annual temperature, second (PC 2) mainly to precipitation during wet or warm periods and third (PC 3) to precipitation during drought (Dupin et al., 2011).

	Predictors	Estimate	Standard error	z-value	Significance level
C-score	Monoploid genome size	0.907	0.150	6.035	***
	Climate PC1	-0.049	0.062	-0.802	ns
	Climate PC2	-0.141	0.058	-2.401	*
	Climate PC3	0.005	0.028	0.167	ns
S-score	Monoploid genome size	-965	0.085	-11.356	***
	Climate PC1	0.056	0.044	1.256	ns
	Climate PC2	0.094	0.043	2.195	*
	Climate PC3	0.011	0.022	0.484	ns
R-score	Monoploid genome size	16.926	5.404	3.133	**
	Climate PC1	-1.072	0.482	-2.225	*
	Climate PC2	-0.494	0.488	-1.011	ns
	Climate PC3	-0.409	0.218	-1.876	ns

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, non-significant.

(Fisher–Pitman permutation test, $p < 0.05$; Figure 4A,B, Table S4). Although not statistically different from its European source populations, the invasive North American populations showed a tendency towards higher C- and S-scores and lower variation ($p > 0.05$; Figure 4). R-scores showed no significant difference among the three groups ($p > 0.05$; Figure S4, Table S4). The multivariate analysis further confirmed the significant differences of adaptive strategies between the haplotype M and the North American native populations, but no difference existed between the two haplotype M populations (Table S5).

4 | DISCUSSION

Using a cosmopolitan model species (*Phragmites australis*), we found extensive intraspecific adaptive strategy variation characterized mainly by divergence between C- and S-selection. We further observed significant latitudinal clines for either C-, S- or R-selection, which were associated with either monoploid genome size (i.e. DNA content in a single chromosome set) or habitat climatic characteristics and most likely due to climatic adaptation on the global scale.

Species-level trait–environment relationships currently form one of the central topics in functional ecology, functional biogeography and evolutionary ecology (Bruehlheide et al., 2018; Violle et al., 2007, 2014). Studies reveal strong associations between intraspecific trait variations and environmental parameters resulting from genetic adaptation to large-scale gradients, for example, latitude or altitude (Cronin et al., 2015; Kühn et al., 2021; Maron et al., 2004; May et al., 2017; Ren et al., 2020; Yang et al., 2021). However, rather than reacting via single trait differences, the adaptation of species usually involves trade-offs between key traits to achieve a higher fitness in a specific environment, which can be summarized by the Grime's adaptive strategies (Grime, 1974, 1977; Grime & Pierce, 2012). Indeed, recent studies have found wide intraspecific strategy variation from R- to S-scores and a

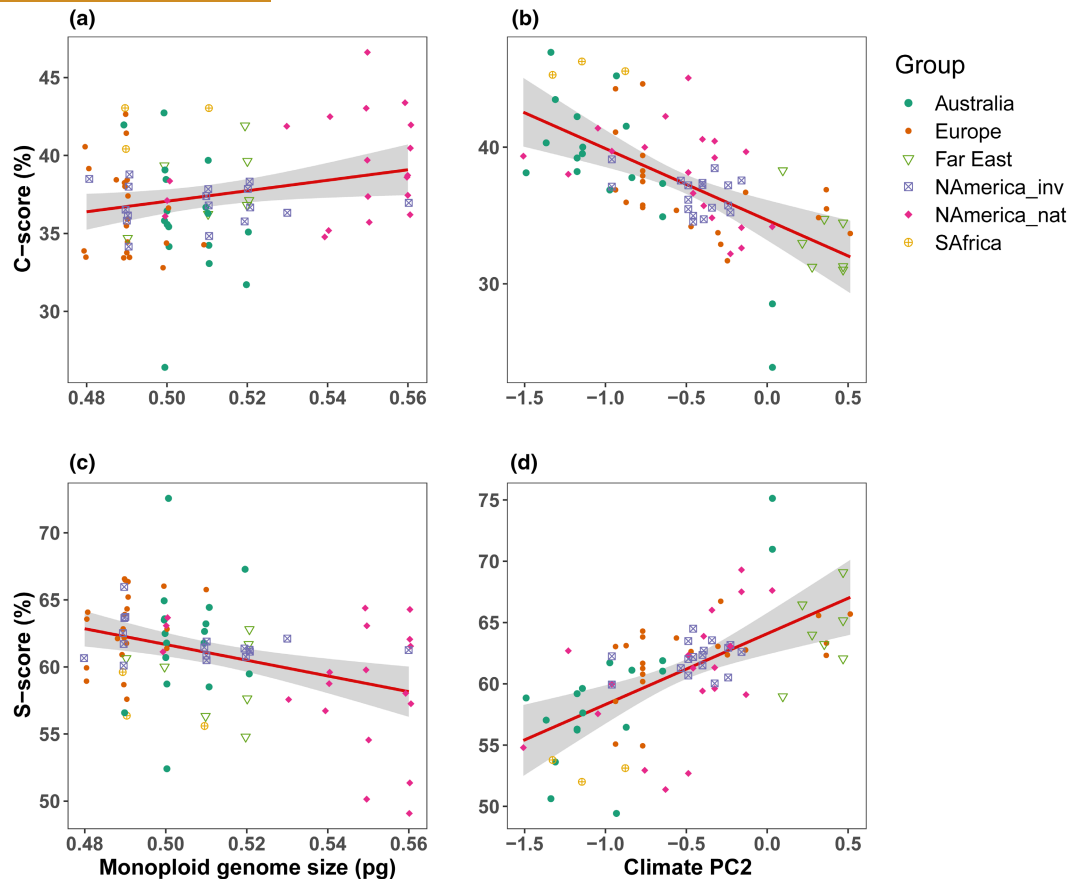


FIGURE 3 Partial regressions of the significant predictors of (upper column) C-scores, and (lower column) S-scores of *Phragmites australis* populations: (a and c) monoploid genome size, and (b and d) Climate PC2: Precipitation during wet or warm periods. Symbols and colours indicate different phylogeographic groups (NAmerica_inv, North American invasive; NAmerica_nat, North American native; SAfrica, South Africa). Detailed statistical results are shown in Table 1. To improve visualization, (a) and (c) were jittered.

latitudinal gradient of adaptive strategies for the model species *A. thaliana* stemming from the adaptation to climate (May et al., 2017; Vasseur et al., 2018). Here, we also found significant latitudinal clines for the three strategy scores for *P. australis* genotypes collected worldwide and cultivated in a common garden, implying local adaptation of genotypes to the climate in regions where they originated (i.e. genotypic differences represent ecotypic differences; Bowen et al., 2017; Meyerson, Cronin, Bhattarai, et al., 2016). Specifically, *P. australis* genotypes originating from places with a high annual mean temperature and more precipitation in the warm and wet seasons tend to be more stress tolerant (Figure 3). As a wetland plant, *P. australis* can survive across a wide range of environments from inland freshwater to tidal brackish marsh (Clevering et al., 2001; Lambertini et al., 2020; Meyerson et al., 2000; Pagter et al., 2005), and tolerate a pH range of 2.5–9.8 (Packer et al., 2017) and widely fluctuating hydroperiod (Englone & Papp, 2006; Pagter et al., 2005), mostly due to its hollow stems and large rhizome systems providing sufficient internal oxygen translocation (Brix et al., 1992, 1996; Pagter et al., 2005). It has been reported that *P. australis* originating from lower latitudes can grow taller but produce fewer shoots and shorter rhizomes than populations from higher latitudes (Clevering et al., 2001;

Meyerson, Cronin, Bhattarai, et al., 2016; Pyšek et al., 2019). This is in accordance with our finding of the decreasing competitive ability of *P. australis* populations along the latitudinal gradient, probably due to the adaptation to precipitation in the warm and wet seasons (Figure 3b).

In addition to the evident associations with climate, we also found that intraspecific strategy scores were strongly linked to the monoploid genome size: genotypes with smaller genome sizes are more stress-adapted than genotypes with larger genomes, which are more competitive (Figure 3), in accordance to the findings of a recent global species-level synthesis (Guo et al., 2024). It has been demonstrated that genome downsizing contributes to the success and rapid spread of flowering plants globally (Benton et al., 2022; Pyšek et al., 2023; Simonin & Roddy, 2018), as small genome size (either holoploid or monoploid) enables having small cells (Pyšek et al., 2018; Simonin & Roddy, 2018), thus leading to faster growth rates (Fridley & Craddock, 2015; Knight et al., 2005; Meyerson, Cronin, Bhattarai, et al., 2016; Suda et al., 2015). Although the fast growth rate is one of the characteristics of strong competitors (Grime, 1974, 1977; Grime & Pierce, 2012; Pierce et al., 2017), here we found that *P. australis* genotypes with bigger monoploid genome sizes are more competitive. The reason could be that the

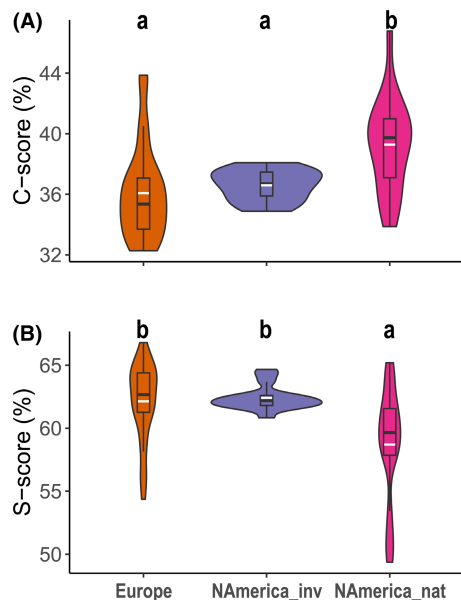


FIGURE 4 Violin plots and boxplots showing the distribution of (A) C-score and (B) S-score of the two haplotype M (Europe and NAmerica_inv) and the native North American populations of *Phragmites australis*, with the white line indicating the mean value for each group. Different letters indicate significant differences among groups ($p < 0.05$), and 'a' indicates the smallest group. Significant test was carried out using the K-sample Fisher–Pitman permutation test (100,000 permutations) using the method 'Tukey' for the multiple post hoc tests. Detailed statistical results are shown in Table S4.

evolution of genome size was affected by the environment (Bureš et al., 2024; Cang et al., 2024). It has been shown that stressful conditions (e.g. high temperature seasonality or elevations) are favourable for smaller genome size populations (Bureš et al., 2024; Cang et al., 2024; Guo et al., 2024; Qiu et al., 2019). As a stress tolerator growing in conditions with fluctuating resources, *P. australis* could benefit more by adapting to varying stressful environments than to be more 'competitive', probably via the huge size of rhizomes (Packer et al., 2017; Pyšek et al., 2019). Another possible reason can be the higher phenotypic plasticity of *P. australis* genotypes with smaller genome sizes revealed from a recent greenhouse experiment (Meyerson et al., 2020).

We found significant adaptive strategy differences between the invasive and native North American lineages, the invasive alien lineage being more stress adapted than the native lineage, in contrast to previous general findings that stress-tolerant species are less likely to be naturalized (Guo et al., 2019; Guo, van Kleunen, et al., 2018). In other words, *P. australis* represents one of the rare cases in which S-selection is a selective advantage during invasion, as supported by an intraspecific competition experiment using the three groups (Pyšek et al., 2020). Yet, it needs to be borne in mind that these findings concern the intraspecific level and that at the species level where comparisons are routinely made, *P. australis* is a competitive stress-adapted species. The capacity of the S-selected genotypes, relative to

other genotypes, to invade seems counterintuitive but only if it is assumed that 'invasion' is a process of direct competition. In environments such as a tropical moist broadleaf forest, the capacity of S-selected species to dominate the canopy is related to the conservative life-time resource use and longevity of these robust perennial plants and the ability of S-selected individuals to outlive C-selected plants and thus persist (Pierce et al., 2017). Indeed, *P. australis* has a potentially long-lived perennial, rhizomatous life form with relatively flexible reproduction capacities, for example, via large amounts of seeds, rhizomes and stolons (Packer et al., 2017). It should also be considered that coexistence typically emerges from a balance between species that differ not only in terms of primary adaptive strategies (resource use, life history) but also in subsets of specific traits that are less important for day-to-day life but come into play during specific events during the life cycle—for instance, pollination syndrome, seed shape, etc. (Grime & Pierce, 2012; Pierce et al., 2014). Where coexisting species exhibit a high degree of phylogenetic divergence, these subtle 'proximal' traits are likely to be critical for coexistence and persistence, but in the case of the co-occurrence of genotypes of a single species, many proximal traits will be identical or similar (especially for a species that can spread and persist by using vegetative reproduction); here, the divergence in the primary CSR strategy represents a functional difference that confers an adaptive advantage. In the case of intraspecific (genotypic) differences in *P. australis*, it is likely that genotype displacement during invasion represents a simple difference in speed and perseverance (the tortoise and the hare) rather than the bewildering array of dominant/subordinate strategies and proximal traits characterizing the dynamics of species assemblages (e.g. Pierce et al., 2014). Thus, although S-selection is known to be relatively rare for alien species that become naturalized (Guo et al., 2019; Guo, van Kleunen, et al., 2018), intraspecific interactions during invasion potentially represent a different and simpler process of 'genotype substitution' or functional displacement.

In contrast to other studies which found significant differences between the two groups of the invasive haplotype M lineage (occurring in Europe and in the introduced range in North America) in genetic characteristics (Guo, Lambertini, et al., 2018), functional traits (Guo et al., 2014; Pyšek et al., 2018) and genome size (Pyšek et al., 2018), here we found that both C- and S-scores for the two groups were not significantly different (Figure 4, $p > 0.05$). Although the invasive genotypes were sampled across the North American continent (Figure S1), they mostly clustered in the CSR triangle together with their European ancestors (Figures 1 and 4), which might be a result of a founder effect or selection in the early stage of the invasion (Guo, Lambertini, et al., 2018). It has been demonstrated that the climatic niches of the two haplotype M groups have shifted; in North America, temperatures show greater fluctuation and precipitation is higher than in Europe (Guo et al., 2013), which is also shown in Figure 3. This heterogeneous environment largely caused the differences in allelic frequencies between the two groups, and human-assisted dispersal further shaped the genetics of *P. australis*

in the introduced range (Bart et al., 2006; Brisson et al., 2010; Guo, Lambertini, et al., 2018). In addition, multiple introduction events (Kettenring et al., 2012; Meyerson & Cronin, 2013), evolution in genome size (Bureš et al., 2024; Pyšek et al., 2018) and differences in traits (Guo et al., 2014; Pyšek et al., 2018) between the two groups all suggest that adaptive evolution occurred, and the North American genotypes are shifting away from their ancestors since the introduction about 150 years ago (Guo, Lambertini, et al., 2018; Meyerson, Cronin, & Pyšek, 2016; Saltonstall, 2002).

5 | CONCLUSIONS

By using a cosmopolitan plant species *Phragmites australis*, our study extends the knowledge of intraspecific variations in Grime's adaptive strategy to study adaptive evolution along environmental gradients. While C- and R-selection are important determinants of invasion success where alien species encounter other species (i.e. interspecific interactions; Guo et al., 2019; Guo, van Kleunen, et al., 2018), S-selection can be an advantage where invasion involves intraspecific 'genotype substitution', probably due to the inherent capacity of these longer lived forms to persist. For *P. australis*, we suggest that its intraspecific strategy variation was shaped by both monoplloid genome size and local climate. The stress-adapted invasive lineage in North America could enlarge its distribution and cause more severe impacts on local ecosystems, which could also be enhanced by the recent climate change (Guo et al., 2013).

AUTHOR CONTRIBUTIONS

W-YG and PP conceived the ideas; CL, HB, LAM and PP designed the experiments and collected the plant materials; JČ, HS and ML collected the data; W-YG analysed the data; W-YG, SP and PP led the writing of the manuscript; all authors contributed to the writing of the drafts and gave final approval for submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The original leaf traits and the calculated CSR scores of each genotype, and the related climate variables and monoplloid genome size values are deposited at https://github.com/wyeco/Paustralis_csr and are mirrored on Zenodo (<https://zenodo.org/records/12508905>).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Information on the genotype used in this study.

Table S2. Rotated component matrix of Principal Component Analysis, adopted from Dupin et al. (2011).

Table S3. Summary of the multivariate analysis (i.e. permutational multivariate analysis of variance) testing the association between the intraspecific adaptive strategy scores of *P. australis* and the genome size and three climate PCs.

Table S4. Summary of the permutational multivariate analysis of variance, as shown in Figure 4 and Figure S4.

Table S5. Summary of the post hoc test of the multivariate analysis (i.e., permutational multivariate analysis of variance) testing the differences between the two haplotype M groups and the native North American native population of *Phragmites australis*.

Figure S1. Locations of the *Phragmites australis* collections. Symbols and colors indicate different phylogeographic groups.

Figure S2. Latitudinal clines in R-scores of *Phragmites australis*. Symbols and colors indicate different phylogeographic groups.

Figure S3. Partial regressions of the significant predictors of R-scores of *Phragmites australis* populations: (a) monoploid genome size, and (b) Climate PC1: mean annual temperature.

Figure S4. Violin plots and boxplots showing the distribution of R-score of the two haplotype M groups and the native North American native population of *Phragmites australis*, with the white line indicating the mean value for each group.

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