

Rapid evolution of latitudinal clines in growth and defence of an invasive weed

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Summary

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- Re-establishment of heritable latitudinal clines in growth-related traits has been recognised as evidence for adaptive evolution in invasive plants. However, less information is known about latitudinal clines in defence and joint clinal evolution of growth and defence in invasive plants.
- We planted 14 native Argentinean populations and 14 introduced Chinese populations of *Alternanthera philoxeroides* in replicate common gardens in China. We investigated the latitudinal clines of traits related to growth and defence, and plasticity of these traits in relation to experiment site and soil nitrogen.
- We found that chemical defence decreased with latitude in introduced populations but increased with latitude in native populations. For growth rate, latitudinal clines were positive in introduced populations but nonexistent in native populations. There were also parallel positive latitudinal clines in total/shoot biomass and specific leaf area. Experiment site affected the occurrence or magnitude of latitudinal clines in growth rate, branch intensity and triterpenoid saponins concentration. Introduced populations were more plastic to experiment site and soil nitrogen than native populations.
- We provide evidence for rapid evolution of clines in growth and defence in an invasive plant. Altered herbivory gradients and trade-off between growth and defence may explain nonparallel clines between the native and introduced ranges.

Introduction

Biological invasions provide useful natural experiments that help to advance our understanding of ecological and evolutionary processes (van Kleunen *et al.*, 2018). More and more empirical examples have shown that rapid adaptation to local environments can promote the establishment and spread of invasive species (Huey *et al.*, 2000; Colautti *et al.*, 2009; Colautti & Barrett, 2013), and that the adaptive process can be repeated for a single species that has invaded distinct regions of the globe (Latimer *et al.*, 2019; van Boheemen *et al.*, 2019a). Therefore, biological invasions provide good opportunities to study contemporary evolution (Colautti & Lau, 2015).

Geographical clines have drawn extensive attention in plant science, because climate is known to be a strong selective factor on plant traits (Daday, 1965; Woods *et al.*, 2012). Latitudinal gradients in abiotic factors, such as temperature, precipitation

and growing season length, could select for genetically based intraspecific clines in plant traits (e.g. Daday, 1954, 1958; Neuffer & Hurka, 1986; Chapin *et al.*, 1993). Some studies have also shown that invasive plant populations have rapidly evolved latitudinal clines in growth and fitness-related traits, either parallel to (Maron *et al.*, 2004, 2007; van Kleunen & Fischer, 2008; Etterson *et al.*, 2008; Dlugosch & Parker, 2008; van Boheemen *et al.*, 2019a) or different from those of native conspecifics (Alexander *et al.*, 2012; Liu *et al.*, 2020b). Currently, we lack a general understanding of why parallel clines sometimes evolve and sometimes do not (Liu *et al.*, 2020b).

Of increasing interest are the effects of species interactions on biogeographic clines. For plant–herbivore interactions, the latitudinal herbivory-defence hypothesis (LHDH) has predicted that herbivory damage and plant defence increase toward lower latitudes where growing seasons are longer and winter conditions are benign (Schemske *et al.*, 2009; Johnson & Rasmann, 2011;

Anstett *et al.*, 2016b). In a meta-analysis, Moles *et al.* (2011) reported that 37% and 21% of the studies that tested the latitude gradients in herbivory or defence showed negative and positive relationships between latitude and herbivory respectively. In total, 51% of the studies showed positive or negative latitudinal gradients in defence levels. Therefore, although support for LHDH is mixed, latitudinal gradients in herbivory and defence seem to be common for native species (Bhattarai *et al.*, 2017). An alternative pattern of geographic variation in herbivory is the 'range-centre' hypothesis (Garcia *et al.*, 2000; Alexander *et al.*, 2007). It proposed that higher abundances of focal plants and greater herbivore pressure at range centres may result in stronger plant–herbivore interactions compared with range edges (Alexander *et al.*, 2007; Woods *et al.*, 2012). In contrast with the prediction of LHDH, the 'range-centre' hypothesis predicts a hump-shaped relationship between latitude and herbivory and defences.

The geographic pattern of plant–herbivore interactions may change during the invasion and spread of introduced plants, which may result from altered herbivore communities and not enough time for introduced populations to evolve gradients (Bezemer *et al.*, 2014; Cronin *et al.*, 2015; Bhattarai *et al.*, 2017). For example, Cronin *et al.* (2015) found latitudinal gradients in herbivore pressure for native genotypes of *Phragmites australis* in the field but no latitudinal gradients among invasive genotypes. Our field survey found that the herbivore damage decreased with latitude for native populations of *Alternanthera philoxeroides* in Argentina (supporting the 'LHDH'), but peaked at the range centre for introduced populations in China (supporting the 'range-centre' hypothesis), and that some defence traits varied with latitude either linearly or quadratically (unpublished data). Significantly, the field survey did not allow us to assess the genetic and plastic contributions to the geographic variation in *A. philoxeroides*–herbivore traits. Also, such evolution of geographic pattern in traits related to plant–herbivore interactions has seldom been tested in invasive plants (but see Bhattarai *et al.*, 2017; van Boheemen *et al.*, 2019b).

Plant growth and defence strategies are evolutionarily intertwined (Woods *et al.*, 2012). Defence may be costly, and allocation to defence is predicted to trade-off with allocation to growth (Coley, 1983; Stamp, 2003). For invasive plants, the evolution of increased competitive ability (EICA) hypothesis predicts that invasive populations may allocate more resource to growth or reproduction, but less to defence due to release from natural enemies (Blossey & Notzold, 1995). Therefore, altered herbivory between native and introduced ranges may result in the evolution of growth and defence simultaneously. It can be inferred that altered herbivory gradients in plants' introduced ranges may influence clinal evolution of not only defence but also growth that may lead to nonparallel clines in growth and defence between native and introduced populations (Bhattarai *et al.*, 2017).

Phenotypic plasticity, the capacity of organisms to express different phenotypes in different environments without genetic modification, may also vary with latitude. According to the climate variability hypothesis (Janzen, 1967; Stevens, 1989), individuals may show higher phenotypic plasticity when

climatic variability increases, such as the increase in temperature variability with latitude. Some empirical studies have also found positive relationships between phenotypic plasticity and latitude in invasive species (Molina-Montenegro & Naya, 2012; Li *et al.*, 2016) and the plasticity of latitudinal clines to experiment site (Maron *et al.*, 2004; Colautti *et al.*, 2009; Woods *et al.*, 2012; Bhattarai *et al.*, 2017). Moreover, introduced populations may have evolved greater plasticity than native populations (Bossdorf *et al.*, 2005; Richards *et al.*, 2006). Therefore, the plasticity of plant traits and latitudinal clines in plant traits may differ between native and introduced populations.

Here, we assessed genetically based clines in growth- and defence-related traits and their plasticity in *Alternanthera philoxeroides* using glasshouse common garden experiments with 28 populations spanning a 13.5 latitudinal range, with 14 populations collected from their native range in Argentina and 14 from their introduced range in China. Our field survey showed slight positive latitudinal clines in nitrogen availability in the native and introduced ranges of *A. philoxeroides* (unpublished data). Furthermore, common garden experiments showed that soil nitrogen addition had strong effects on the growth- and defence-related traits of *A. philoxeroides* (Wang *et al.*, 2015; Zhou *et al.*, 2017). Therefore, we measured quantitative traits related to growth and defence under two levels of soil nitrogen availability considering it may affect the expression of latitudinal clines as found for experiment sites. Experiments were replicated at two sites, located at the northern and southern edges of the species' range in China. We asked the following questions:

- (1) Do genetically based latitudinal clines in plant traits related to growth and defence of introduced populations parallel those of native populations?
- (2) Do latitudinal clines of plant traits in native and introduced populations differ between experiment sites?
- (3) Are introduced populations more plastic to experiment site and soil N availability?

We hypothesised that:

- (1) There are nonparallel clines in growth and defence traits between native and introduced populations.

For native *A. philoxeroides* populations, there may be negative genetically based latitudinal clines in defence (according to the LHDH). The introduced populations may not have sufficient time to evolve latitudinal clines or have evolved hump-shaped defences in response to higher herbivore pressure at range centre than range margins (according to 'range-centre' hypothesis). Introduced populations at high latitude that experienced enemy release might have evolved increased growth as predicted by the EICA hypothesis, but this may not occur for low- or middle-latitude populations which have received biocontrol and suffered from even higher herbivore pressure than native populations (Lu *et al.*, 2013; unpublished data).

- (2) Experiment site affects the latitudinal clines of plant traits in native and introduced populations.
- (3) Introduced populations are more plastic to experiment site and soil N availability than native populations.

Materials and Methods

Study species and sample collection

Alternanthera philoxeroides (Mart.) Griseb (alligator weed) is a stoloniferous perennial herb from the Amaranthaceae family that can thrive in both terrestrial and aquatic habitats. It is native to South America and has become a noxious weed worldwide. *Alternanthera philoxeroides* was introduced to China in the 1930s as forage for livestock and is now widely distributed from 21°N to 36.8°N in China (Lu *et al.*, 2013). It propagates mainly by vegetative means (stems or storage shoots) in China, and overwinters by storage roots (Pan *et al.*, 2007). Random Amplification Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) analyses suggest the Chinese populations might have resulted from a single introduction with low levels of genetic diversity (Xu *et al.*, 2003; Geng *et al.*, 2016). Amplified Fragment Length Polymorphism (AFLP) analysis of Chinese and Argentinian populations suggests that Chinese populations were most closely related to the population from Jujuy (65.35°W, 24.08°S) in Argentina (Li, 2018).

From 24 July to 13 August 2018, we collected stem fragments and storage roots of *A. philoxeroides* at 14 sites along the latitudinal range in China (Fig. 1; Supporting Information Table S1). At each site, we chose three subpopulations of *A. philoxeroides* that were at least 100 m apart or geographically separated (by road, river etc.) from each other and randomly collected five propagules from five individuals in each subpopulation. The minimum distance between collected plants was 10 m to ensure different individuals. Native populations were collected from 14 sites in Argentina in the same way during 26 January to 26 February 2019 (Fig. 1; Table S1). The sampled stem fragments and storage roots were transplanted in a glasshouse on the Jiangwan campus of Fudan University (Shanghai, China), and vegetatively propagated with new stems (with nodes) three times to remove maternal effects before experiment set-up.

Experimental set-up

We established replicate experiments at the Institute of Forestry and Garden Science in Zengcheng Guangzhou (23.29°N, 113.87°E, Fig. 1) and the campus of Ludong University in Zhifu Yantai (37.83°N, 121.37°E; Fig. 1). Both experiment rooms were semiopen glasshouses where rain could not reach but air was exchanged with the outside.

On 10 June 2019, in the Fudan University glasshouse, we selected two maternal families randomly from each subpopulation collected from each latitudinal site. For each latitudinal population, six maternal families from three subpopulations were used for the subsequent experiment. We cut eight stem fragments (with one node, *c.* 2 cm in length) from each maternal family (altogether (14 native populations + 14 introduced populations) × 6 maternal families × 8 = 1344 stem fragments including all 14 native and 14 introduced populations). To promote shoot and root development, all stem fragments were put on moist tissue papers in a 25°C : 28°C, 12 h : 12 h, dark : light incubator. After

2 wk, for each maternal family, we selected two plantlets randomly (out of eight) with similar height and two pairs of leaves, and transplanted 336 ((14 native populations + 14 introduced populations) × 6 maternal families × 2) plantlets separately into 0.4 l plastic pots with a 1 : 1 mixture of sand and fine vermiculite in the glasshouse in Guangzhou. We arranged all pots randomly on 16 tables with at least 20 cm between pots to avoid above-ground competition. For plants subsequently used to measure constitutive defence levels, we put all pots into 16 nylon insect screens (40 mesh, 1.6 m × 1.6 m × 0.8 m) supported by PVC tubes to avoid herbivory. At 3 d after transplanting, one individual from each maternal family was assigned randomly to the low nitrogen treatment and the remaining one assigned to the high nitrogen treatment according to a fully randomised experimental design. Six replicate plants for each origin × latitude × N treatment were used. Next, 10 ml NH₄Cl solution (N concentration: 500 mg l⁻¹) and 90 ml water were added to the pots in the low nitrogen treatment and 100 ml NH₄Cl solution at the same concentration to pots in the high nitrogen treatment at weekly intervals. To meet the plants' requirements for other nutrients, we added 50 ml 400% Hoagland solution (N free) to each pot on the third day after nitrogen treatment every week. A tray (2 cm high) was put under each pot to avoid the loss of fertiliser solution and cross-contamination. During the experiment, we watered each plant with 50 ml water when the soil looked dry. All pots were re-randomised every week to reduce the effect of location in the glasshouse and insect screens.

The above process was repeated for the experiment in Yantai. On 3 July 2019, we collected stem fragments from the same family of each subpopulation used in the Guangzhou experiment. We transplanted 336 individuals to the glasshouse in Yantai on 13 July 2019. Pots, soil and treatment were consistent with that applied in Guangzhou with one exception: due to the different size of glasshouse tables, insect screens were 0.8 m × 0.8 m × 0.5 m in Yantai. The semiopen experiment rooms located at low and high latitudes differed in temperature, relative humidity and duration of sunshine. In Guangzhou, the average temperature and relative humidity were higher, but duration of sunshine was lower during the experiment (Table S2).

Measurements

As the experiment went on, we recorded the length of the main stem and the number of branches every week. We harvested all plants in Guangzhou on 16 August 2019 and Yantai on 7 September 2019 (8 wk after transplanting). We separated the plants into above-ground and below-ground parts. Below-ground parts were carefully washed and then divided into the fine roots and storage roots. Above-ground parts of a plant were divided into one main stem, leaves and branches. The length of main stems was measured by rulers. A 0.5-cm-diameter leaf disc was removed from the first fully expanded leaf of the main stem and photographed to determine trichome density by counting the number of trichomes on the top and bottom surfaces. The pairs of leaves on the second nodes were flattened and photographed to calculate the leaf area using the Digimizer Image Analysis

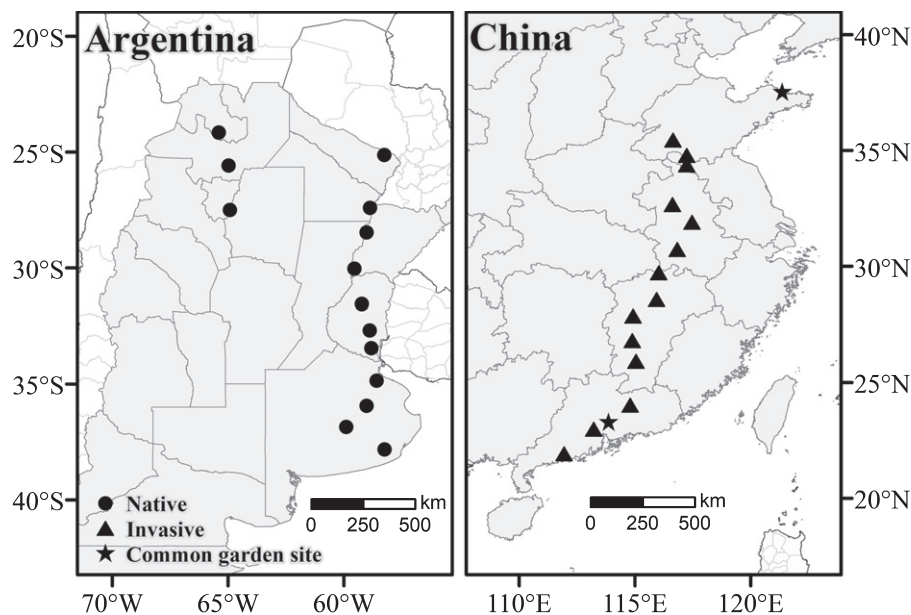


Fig. 1 Map of the locations of *Alternanthera philoxeroides* source populations used in this study and the locations of the common gardens at Guangzhou (south) and Yantai (north) in China.

Software (v.4.3). Next we dried all plant materials at 60°C for 72 h before determination of dry mass. Growth rate was estimated by the early elongation rate of stem (mm d^{-1} , average daily increase in length). Specifically, we calculated the elongation rate of the main stem each week after transplanting and chose the rate at the fastest growing stage (the third week) on average for all plants. We chose this early growth stage because pot size was unlikely to be limiting growth factor and few branches had developed, both of which should make the stem elongation rate in this stage more predictive for the growth rate of whole plants. Functional traits included specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$, ratio of leaf area and leaf dry weight), branch intensity (BI, ratio of branch dry weight and main stem dry mass), specific stem length (SSL, cm g^{-1} ; ratio of length of main stem and main stem dry mass) and root:shoot ratio (RSR; ratio of below-ground biomass and above-ground biomass). Constitutive defence traits included trichome density (ratio of trichome number and leaf area, number cm^2) and the concentration of total triterpenoid saponins in leaves (mg g^{-1}). Triterpenoid saponins are among the primary defensive chemicals produced by *A. philoxeroides* (Faizal & Geelen, 2013). Our previous common garden experiments found that both trichome density and concentrations of triterpenoid saponins negatively correlated with herbivore performance and were inducible by herbivory in *A. philoxeroides* (Liu *et al.*, 2018, 2020a). We pooled dry leaves of two replicate individuals from the same family under the same nitrogen treatment in each garden and used UV spectrophotometry to measure the concentration of total triterpenoid saponins. (Wang *et al.*, 2017; Liu *et al.*, 2018).

Statistical analyses

To test whether native and introduced populations of *A. philoxeroides* exhibited genetically based latitudinal clines and estimate the plasticity of plant traits and latitudinal clines to garden site and soil N availability in biomass (total biomass, shoot

biomass and storage root biomass), growth rate (early elongation rate of stem), functional traits (SLA, BI, SSL, and RSR) and defence traits (trichome density and total triterpenoid saponins), we used linear mixed models with the LME4 package (Bates *et al.*, 2015) in R v.3.5.1 (R Core Team, 2018) based on all the data from two gardens (the analysis did not include data from the individuals that had been accidentally fed on by insects or died, and 300 and 326 observations were effective for each trait from Guangzhou and Yantai, respectively). RSR data were natural log-transformed and trichome density data were square root transformed to meet the assumptions of a Gaussian distribution of the residuals. We included origin (native vs introduced), latitude (latitude of collection site), garden (experiment site, Guangzhou vs Yantai), N (low nitrogen vs high nitrogen), and their interactions as fixed effects. Random effects included subpopulations nested in latitude and maternal family nested in subpopulations. We initially included a quadratic term (latitude^2) as a fixed effect to test for nonlinear variation in traits with latitude, but this was not significant for any trait, therefore we dropped this term in subsequent analyses. The significance of fixed effects in the mixed models was assessed by Type II Wald chi-squared test using the CAR package (Fox & Weisberg, 2019). Tukey honest significant difference (HSD) post-hoc analyses using the EMMEANS package (Lenth, 2019) were used to contrast the slope in response to latitude for plant traits in different origins, gardens or N treatments.

To explore whether there was a quantitative trade-off between growth rate and defence traits in native and introduced populations of *A. philoxeroides*, we regressed defence level (trichome density and triterpenoid saponins concentration) on stem elongation rate. Data sets for growth rate and defence traits in each origin (native, introduced) under each soil N treatment (N+, N-) at each experiment site (GZ, YT) were analysed separately. Trichome density data were square root transformed. ANOVA was conducted to test the significance of regression coefficients. We also use multivariate linear regression of total biomass (as an estimate of fitness) on growth rate and defence traits values to

estimate selection gradients (Lande & Arnold, 1983). Similar to the above analysis, data sets were analysed separately according to origin of populations, experiment sites and soil N treatment. The growth rate and defence traits were standardised. We regressed growth rate and defence traits onto total biomass with both linear and quadratic terms and estimated the standardised directional (β) and nonlinear (γ) selection gradients.

To examine the difference in the phenotypic plasticity of native and introduced populations of *A. philoxeroides*, we compared the slopes in the norms of reactions to N and garden in plant traits (Valladares *et al.*, 2006). Specifically, we used Tukey HSD post-hoc tests to examine differences in trait mean values under different N or garden treatments in the mixed model above when native and introduced populations showed different responses to N or garden treatment (significant interactions involving origin and N/garden/N \times garden).

Results

Latitudinal clines in plant traits

Biomass and growth rate Both native and introduced populations showed parallel latitudinal clines in total and shoot biomass (significant latitude effects, nonsignificant origin \times latitude; Table 1), and this relationship was not affected by the experiment site or soil N availability (no significant effects involving latitude \times garden/N; Table 1). Native and introduced populations from higher latitudes had greater biomass (Fig. 2a) and greater shoot biomass (Fig. 2b). There was no latitudinal cline in storage root biomass (no significant effects involving latitude; Table 1, Fig. 2c). Latitudinal clines in stem elongation rate were nonparallel between native and introduced populations (significant origin \times latitude effect; Table 1) and experiment site affected the relationship between elongation rate and latitude (significant garden \times latitude effect; Table 1; Fig. 2d). Introduced populations showed a positive latitudinal cline in stem elongation rate and plants from the Guangzhou common garden exhibited steeper increase in stem elongation rate with increasing latitude than plants from Yantai common garden, but the relationship was not significant for native populations (Fig. 2d).

Functional traits First, native and introduced populations showed parallel latitudinal clines in SLA, which increased with latitude and this relationship was not affected by the experiment site or soil N availability (Table 1; Fig. 2e). Second, BI was nonparallel between native and introduced populations, and the experiment site affected the relationship between BI and latitude (Table 1). In Guangzhou, the BI of both native and introduced populations increased with latitude (Fig. 2f). In Yantai, the trend was not significant in introduced populations but significant in native populations (Fig. 2f). Third, the SSL and root : shoot ratio (RSR) did not vary with latitude in Guangzhou for native and introduced populations (Fig. 2g,h). However, in Yantai, both traits (SSL and RSR) increased with latitude for native populations but did not vary with latitude for introduced populations (Fig. 2g,h).

Table 1 The effects of origin (O), latitude (L), soil N (N), garden (G), and their interactions on plant traits of *Alternanthera philoxeroides*.

Variable	Biomass			Growth trait			Functional trait			Defence trait		
	Total χ^2 (df)P	Shoot χ^2 (df)P	Storage root χ^2 (df)P	Elongation rate χ^2 (df)P	SLA χ^2 (df)P	BI χ^2 (df)P	SSL χ^2 (df)P	RSR χ^2 (df)P	Trichome density χ^2 (df)P	Triterpenoid saponins χ^2 (df)P		
Origin	65.77 (1)***	37.70 (1)***	42.41 (1)***	12.63 (1)***	42.27 (1)***	47.25 (1)***	4.65 (1)*	32.90 (1)***	43.73 (1)***	8.43 (1)**		
Latitude	12.07 (1)***	11.56 (1)***	1.98 (1)	5.60 (1)*	16.53 (1)***	20.54 (1)***	1.17 (1)	0.15 (1)	0.13 (1)	0.55 (1)		
N	22.42 (1)***	6.15 (1)*	453.37 (1)***	4.15 (1)*	11.33 (1)***	706.11 (1)***	587.73 (1)***	841.75 (1)***	109.92 (1)***	22.46 (1)***		
Garden	39.99 (1)***	50.07 (1)***	16.78 (1)***	67.23 (1)***	0.62 (1)	530.24 (1)***	73.49 (1)***	7.86 (1)**	96.58 (1)***	6.68 (1)**		
O \times L	0.18 (1)	0.55 (1)	1.40 (1)	16.21 (1)***	0.33 (1)	6.17 (1)*	2.87 (1)	2.16 (1)	0.98 (1)	7.65 (1)**		
O \times N	1.64 (1)	4.44 (1)*	2.86 (1)	1.17 (1)	1.54 (1)	20.99 (1)***	1.16 (1)	0.00 (1)	12.17 (1)***	0.05 (1)		
L \times N	0.01 (1)	0.16 (1)	0.86 (1)	1.67 (1)	1.26 (1)	2.49 (1)	0.04 (1)	0.00 (1)	0.00 (1)	0.08 (1)		
O \times G	2.70 (1)	3.39 (1)	0.36 (1)	0.89 (1)	74.71 (1)***	44.13 (1)***	5.13 (1)*	2.05 (1)	7.33 (1)**	24.13 (1)***		
L \times G	0.34 (1)	0.43 (1)	0.00 (1)	12.45 (1)***	3.46 (1)	8.44 (1)**	2.55 (1)	0.60 (1)	3.06 (1)	8.49 (1)**		
N \times G	26.85 (1)***	43.00 (1)***	6.55 (1)*	42.44 (1)***	81.92 (1)***	187.68 (1)***	151.20 (1)***	40.33 (1)***	1.23 (1)	1.37 (1)		
O \times L \times N	0.23 (1)	0.42 (1)	0.07 (1)	0.67 (1)	0.02 (1)	2.62 (1)	2.18 (1)	0.16 (1)	0.20 (1)	0.01 (1)		
O \times L \times G	0.00 (1)	0.16 (1)	0.37 (1)	11.12 (1)***	1.03 (1)	0.06 (1)	6.60 (1)*	3.99 (1)*	1.24 (1)	0.10 (1)		
O \times N \times G	1.40 (1)	0.60 (1)	5.58 (1)*	1.20 (1)	3.53 (1)	19.38 (1)***	2.99 (1)	1.52 (1)	0.31 (1)	0.13 (1)		
L \times N \times G	0.06 (1)	0.07 (1)	0.00 (1)	0.61 (1)	0.06 (1)	0.04 (1)	0.40 (1)	0.00 (1)	0.05 (1)	0.35 (1)		
O \times L \times N \times G	0.83 (1)	1.49 (1)	0.71 (1)	2.77 (1)	0.19 (1)	3.49 (1)	0.70 (1)	0.95 (1)	0.15 (1)	0.03 (1)		

df, degrees of freedom; χ^2 , chi-squared test statistic.

The functional traits are: BI, branch intensity; RSR, root : shoot ratio; SLA, specific leaf area; SSL, specific stem length.

Statistical significance is indicated as: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

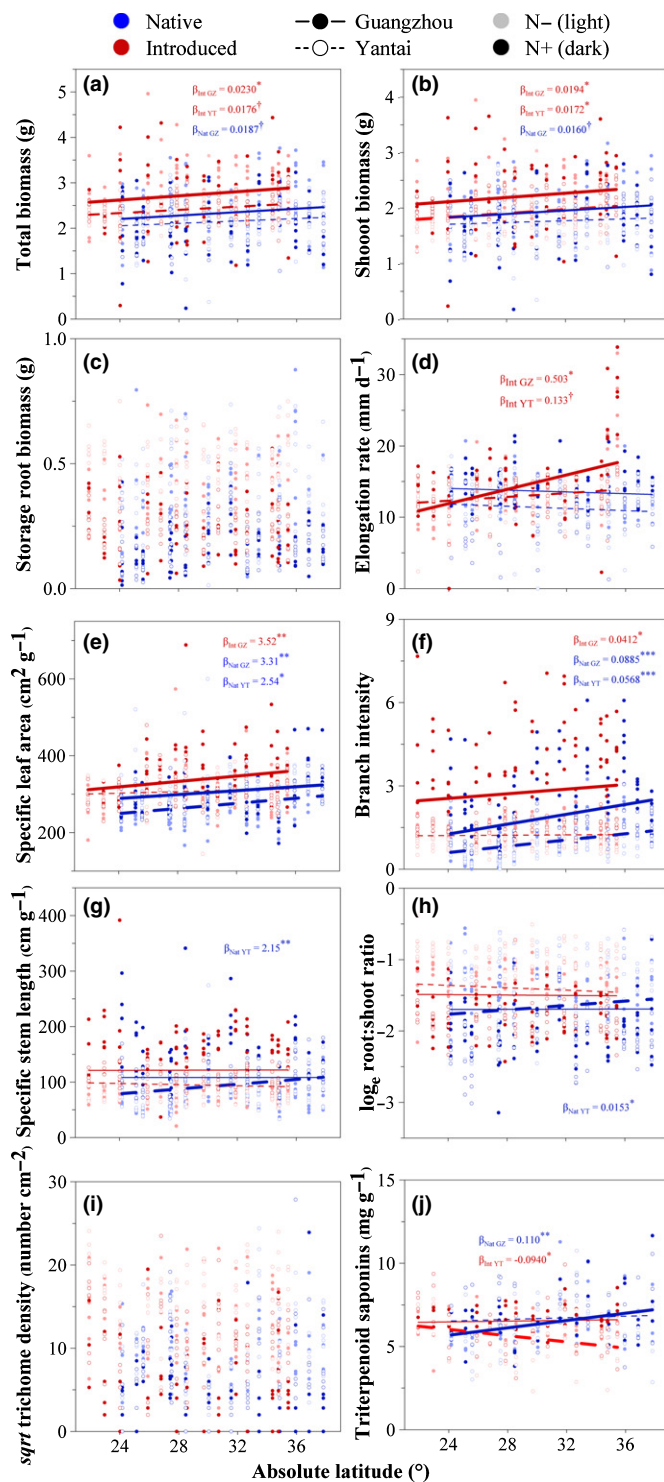


Fig. 2 Effects of origin, latitude, nitrogen (N) addition, and garden site on biomass/growth traits (a–d), functional traits (e–h), and defence traits (i, j) of *Alternanthera philoxeroides*. Points are raw data. The relationship between a trait and latitude is shown by a line only for the χ^2 test results including significant latitude effects or interaction involving latitude. Thick lines denote significant effects of latitude on traits ($P < 0.05$); lines in middle thickness denote marginally significant effects of latitude on traits ($0.05 < P < 0.1$); thin lines denote nonsignificant effects of latitude ($P > 0.1$). Slopes are reported for the significant or marginal significant relationships. The slopes and intercepts of trait–latitude relationships for native and introduced populations were predicted by averaging the effects of N by the mixed model. Significance level of slope of trait–latitude relationship is indicated by: †, $0.05 < P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Yantai, the triterpenoid saponin concentration of introduced populations decreased with latitude, but there was no cline in native populations (Fig. 2j).

Association between growth and defence

There was no association between trichome density and elongation rate for native and introduced populations (Table S3). In Guangzhou, there was no association between triterpenoid saponins concentration and elongation rate for native and introduced populations (Table S3; Fig. 3a,b). In Yantai, there was a marginally significant negative correlation between triterpenoid saponin concentration and elongation rate under low N treatment, and a significant negative correlation under high N treatment for introduced populations, but the correlation was not significant for native populations (Table S3; Fig. 3c,d).

The sign and magnitude of standardised selection coefficients varied among origin of populations and experiment environments (Table S4). There was a sign reversal of the standardised selection gradient (β) between elongation rate and trichome density in native populations under low N treatment in Guangzhou (Table S4). But the direction of selection for elongation rate and trichome density were consistent in plants under high N treatment in Guangzhou, and in introduced populations under high N treatment and native populations under low N treatment in Yantai. There were also sign reversals of the standardised selection gradient between elongation rate and triterpenoid saponins concentration in native populations under high N treatment in Guangzhou and in introduced populations under low N treatment in Yantai (Table S4). Significant quadratic selection coefficients (γ) showed generally disruptive selection for these traits, except for stabilising selection for trichome density in introduced populations under low N treatment and for elongation rate in native populations under high N treatment in Yantai (Table S4).

Phenotypic plasticity of plant traits

Introduced and native populations showed different responses to soil N addition in shoot biomass, BI, and trichome density (significant $O \times N$ interactions; Table 1). Generally, the absolute increase in shoot biomass and BI, and decrease in trichome density in introduced populations were more than those in native populations in response to increased soil N availability (Fig. 4a–c).

Defence traits Native and introduced populations showed no latitudinal clines in trichome density (Table 1; Fig 2i). Clines in triterpenoid saponins concentration were nonparallel between native and introduced populations, and experiment site affected the relationship between triterpenoid saponins concentration and latitude (Table 1). In Guangzhou, native populations exhibited an increase in triterpenoid saponins concentration with latitude, but there was no cline in introduced populations (Fig. 2j). In

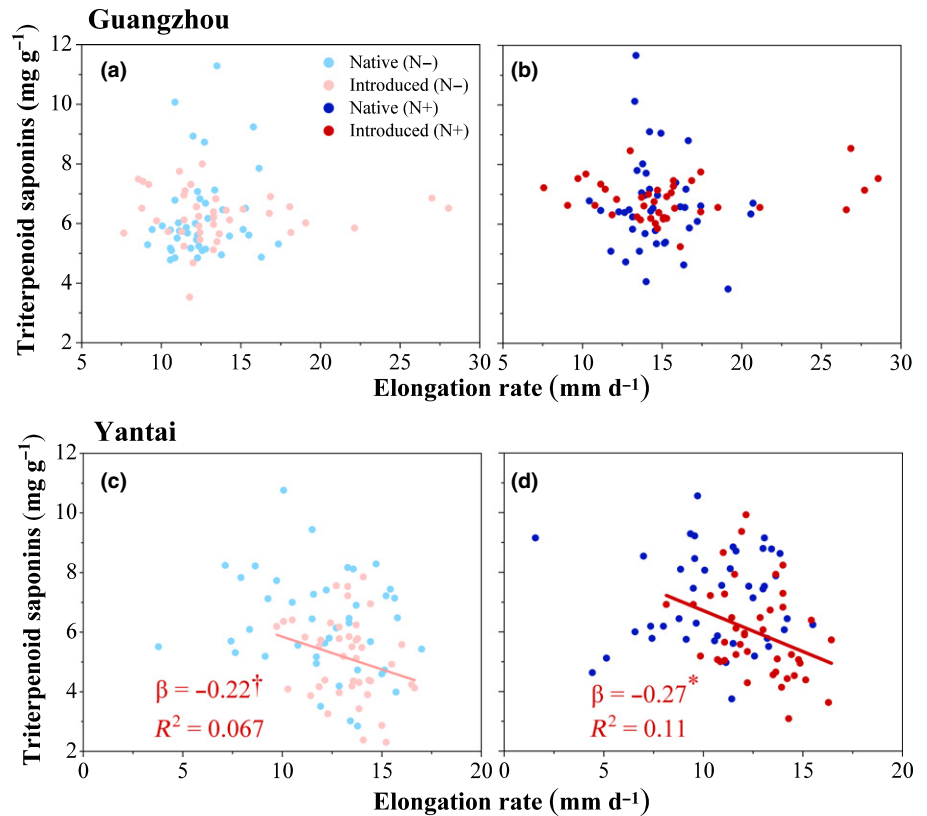


Fig. 3 Triterpenoid saponins concentration of *Alternanthera philoxeroides* responses to elongation rate in the native and invasive populations under two nitrogen (N) treatments (a, c, N-; b, d, N+) in Guangzhou (a, b) and Yantai (c, d). Thick lines denote significant effects of latitude on traits ($P < 0.05$); the line in middle thickness denote marginally significant effects of latitude on traits ($0.05 < P < 0.1$). Significance level of regression coefficient is indicated by: †, $0.05 < P < 0.1$; *, $P < 0.05$.

Introduced and native populations also showed different responses to experiment site in SLA, BI, SSL, trichome density and triterpenoid saponins (significant $O \times G$ interactions; Table 1). For traits other than SLA, the absolute variations in introduced populations were greater than those in native populations when grown in Yantai compared with in Guangzhou (Fig. 4d–h).

The different responses of introduced and native populations to increased soil N availability were affected by experiment site for storage root biomass and BI (significant $O \times N \times G$ interactions; Table 1). The response of storage root biomass to increased soil N in introduced populations was similar to that in native populations in Guangzhou, but stronger in Yantai (Fig. 4i,j). By contrast, the BI in introduced populations showed a stronger response to soil N supply in Guangzhou (Fig. 4k,l).

The significant effects of origin, N and garden site in our models (Table 1) showed that there was genetic differentiation of plant traits between native and introduced populations, and that phenotypic plasticity of alligator weed to experiment site and soil N availability was strong (Fig. S1).

Discussion

Our results showed that growth rate increased with latitude in introduced, but not in native populations, while chemical defence decreased with latitude in introduced populations in Yantai and increased with latitude in native populations in Guangzhou, and that there were parallel clines in some traits related to growth between native and introduced populations. Furthermore, we found that genetically based latitudinal clines in elongation rate,

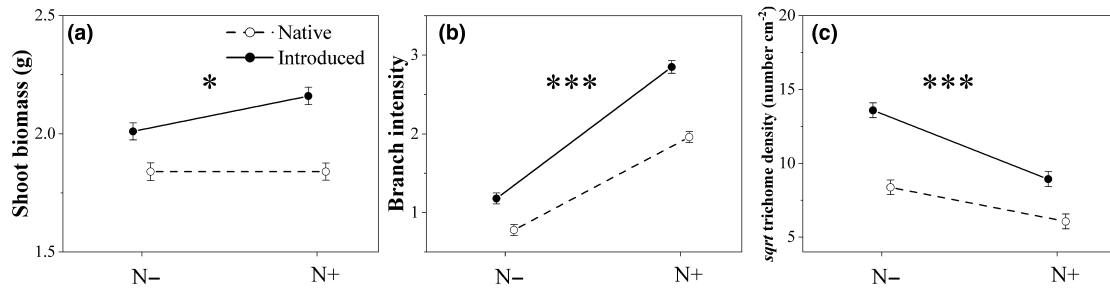
BI, SSL, RSR and triterpenoid saponins showed plasticity to experiment site. The nature of this plasticity also differed between native and introduced populations in elongation rate, SSL and RSR. Finally, we found that introduced populations had greater phenotypic plasticity to soil N availability and experiment site than native populations. Below, we discuss these results in relation to classical biogeographical theories about plant defence and growth (i.e. LHDH, ‘rang-centre’ hypothesis and EICA hypothesis).

First, our observation of a negative latitudinal cline of chemical defence supports the prediction of LHDH, but only in introduced populations. Second, we found no evidence for the ‘range-centre’ hypothesis, as defence did not vary with latitude quadratically. Finally, the EICA hypothesis was supported at high latitudes, where introduced populations had higher growth rates and lower levels of chemical defences compared with native populations. These results suggested that invasive alligator weed might have evolved rapidly in response to abiotic and biotic environment gradients across latitudes and that the genetic and plastic regulation of traits and clines in plant traits might have changed during invasion.

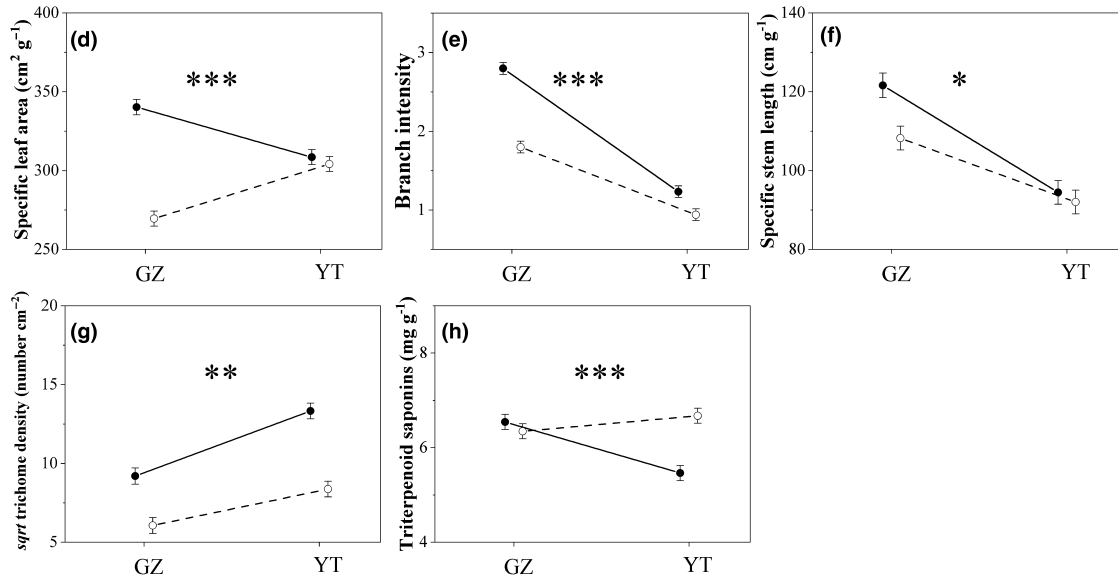
Do genetically based latitudinal clines in plant traits related to growth and defence of introduced populations parallel those of native populations?

Our results showed that there were genetically based latitudinal clines in biomass, growth rate, functional traits and defence traits in introduced populations (Fig. 2). Previous genetic studies of this plant have suggested that the genetic diversity of the introduced populations in China is low and the invasion might have

Origin × N interactions



Origin × Garden interactions



Origin × N × Garden interactions

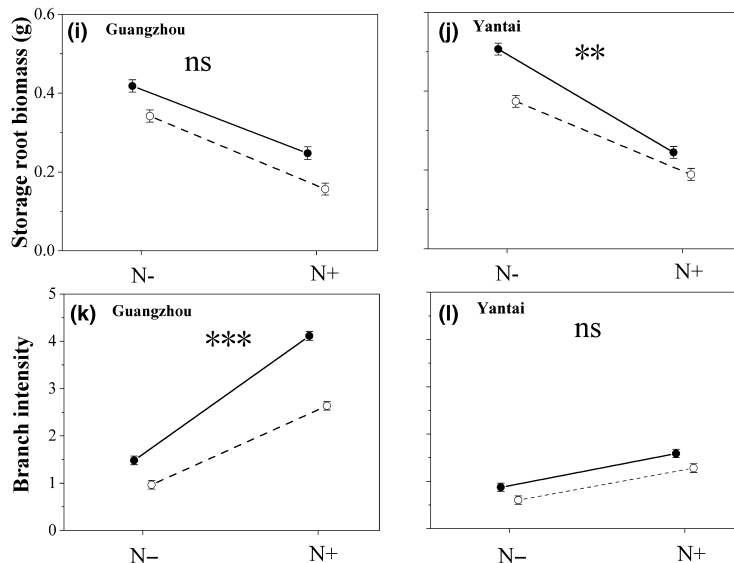


Fig. 4 Interactions between origin and N (a–c) or experiment site (d–h), and origin × N × garden (experiment site) (i–l) on plant traits of *Alternanthera philoxeroides*. Points are the least-squares means (\pm SE) for different origins under different N treatments or/and in different gardens. Significance level of origin × N or origin × garden is indicated by: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

resulted from a single introduction (Xu *et al.*, 2003; Geng *et al.*, 2016). Therefore, the introduction of multiple genotypes may not explain the establishment of latitudinal clines in introduced

populations (Maron *et al.*, 2004). Alternatively, introduced populations might have evolved latitudinal clines rapidly in response to the environmental gradients in the introduced range.

We observed parallel clines in total biomass, shoot biomass and SLA between native and introduced populations (Fig. 2a,b, e). Parallel relationships in clines of fitness or growth-related traits are consistent with most studies which compared latitudinal clines in plant traits between native and introduced populations (e.g. Maron *et al.*, 2004, 2007; van Kleunen & Fischer, 2008; van Boheemen *et al.*, 2019a). For example, Maron *et al.* (2004) found that latitudinal clines in size, fecundity and leaf area of introduced *Hypericum perforatum* broadly converged with clines in native populations. van Boheemen *et al.* (2019a) found parallel adaptations across latitudes in life-history traits between native and introduced ranges of *Ambrosia artemisiifolia*. These results may reflect the rapid evolution of clines in growth-related traits to similar latitudinal gradients of abiotic factors between continents (Maron *et al.*, 2004).

We also observed nonparallel clines in chemical defence and some growth traits. Although the nonparallel clines in chemical defence supported our hypothesis overall, but provided mixed support for the LHDH and 'range-centre' hypotheses. For introduced populations, we found a negative correlation between chemical defence (i.e. triterpenoid saponins) and latitude in the Yantai common garden, which supports the prediction of LHDH. However, our field survey showed that herbivory damage on alligator weed peaked at the range centre (unpublished data), and the abundance of the main herbivore *Agasicles hygrophila* (an introduced specialist biocontrol insect) peaked at middle latitudes (Lu *et al.*, 2013), in accordance with the 'range-centre' hypothesis. These different latitudinal patterns in defence traits and herbivory might have resulted partially from the type of chemical defence compounds (e.g. qualitative or quantitative defences) (Muller-Scharer *et al.*, 2004), and variation in latitudinal patterns of herbivory according to herbivore specialisation (Anstett *et al.*, 2016a). Previously, we found that triterpenoid saponins reduced the performance of a generalist herbivore *Spodoptera litura* but not that of *A. hygrophila* (Liu *et al.*, 2020a). Therefore, the concentration of triterpenoid saponins in alligator weed might have evolved with latitude in response to the latitudinal clines in generalist herbivory, which may be different from those of specialist herbivory (Anstett *et al.*, 2014).

By contrast, for native populations, the positive correlation between chemical defence and latitude was the opposite of that predicted by the LHDH. Positive latitudinal clines in chemical resistance compounds (including tannins, lipids etc.) were also reported in a meta-analysis, and might be explained by the greater cost of losing a given amount of leaf tissue in low-productivity environments (Moles *et al.*, 2011). In our system, another possible explanation for the positive cline in chemical defence is the increased ratio of generalist to specialist herbivores with latitude (Sosa *et al.*, 2004; Forister *et al.*, 2015). The type of herbivory may play an important role in the clinal evolution of this generalist herbivore-related defence trait when the latitudinal cline in total herbivory is relatively weak (unpublished data). In another study in both the field and a common garden, Anstett *et al.* (2014, 2016a) also found that latitudinal gradients in herbivory and defences of *Oenothera biennis* did not always support the LHDH and strongly depended on herbivore specialisation.

These results strongly support the view that every pattern of plant defence (positive, negative and no relationship) is possible across latitudinal gradients, therefore herbivore specialisation needs to be considered when latitudinal gradients in plant defence are tested (Anstett *et al.*, 2016a,b). Therefore, invasive plants may evolve quickly in response to altered latitudinal patterns in herbivory during invasion, and establish latitudinal gradients in defence that are not parallel to those in their native ranges (Bhattarai *et al.*, 2017).

Conversely, nonparallel latitudinal clines in growth rate supported our hypothesis, which is based on the EICA hypothesis and nonparallel herbivory pattern between continents. The direction of evolution in growth rate should be opposite to that in defence according to the EICA hypothesis (Blossey & Notzold, 1995). Our overall clines of growth rate and defence are consistent with those predicted from the hypothesis (Fig. 2d,j), and we also found evidence for a trade-off between growth rate and chemical defence (Fig. 3c,d; Table S4). These results suggested that it is important to consider the variation in herbivory caused by geographical factors when the EICA hypothesis in introduced plants is tested (Colautti *et al.*, 2009; Turner *et al.*, 2014). Nonparallel clines in BI, SSL and RSR that were evident in native populations, but weak or absent in introduced populations, may reflect different selective pressures across ranges or insufficient time for evolution to act in the introduced range (Liu *et al.*, 2020b).

Do latitudinal clines of plant traits in native and introduced populations differ between gardens?

We found significant interactions involving garden and latitude in five out of eight traits with significant latitudinal clines (Table 1), which supported our hypothesis that experiment site affects the latitudinal clines in plant traits. These results indicated that plasticity can regulate the expression of these genetically based latitudinal clines (Richards *et al.*, 2006). The plasticity of latitudinal clines to experimental site varied by origin for stem elongation rate, SSL and RSR, which suggested that plastic regulation of clines has been modified during invasion (Liu *et al.*, 2020b). Such garden effects on latitudinal clines in plant traits seem to be quite common, as they have been observed in multiple common garden studies, demonstrating the need to consider the experiment site in studies of the effect of plant origin on clinal variation (Williams *et al.*, 2008; Colautti *et al.*, 2009).

Are introduced populations more plastic to experiment site and soil N?

Our results showed that introduced populations generally had greater plasticity to soil N availability or experiment site than native populations (Fig. 4), which supported the prediction of greater plasticity of introduced populations than native conspecifics (Richards *et al.*, 2006; Davidson *et al.*, 2011). Such an increase in plasticity to soil N supply or experiment site in introduced populations was also found in invasive *Plantago virginica* and *Phragmites australis* (Bhattarai *et al.*, 2017; Luo *et al.*, 2019).

Bhattarai *et al.* (2017) found that the invasive genotypes of *Phragmites australis* were 2.5 times more plastic to experiment site than native genotypes in traits related to defences, nutritional condition and palatability to herbivores. Luo *et al.* (2019) found greater plasticity of below-ground biomass and leaf area to N supply in invasive than native *Plantago virginica* populations. These results indicated that introduced populations may benefit more from soil N supply and variable climate than native populations. In addition, some significant N \times G even O \times N \times G interactions in our model addressed again that the experiment sites need to be considered carefully for the studies of phenotypic plasticity and evolution of phenotypic plasticity in invasive plants.

It is important to bear in mind that native populations of alligator weed failed to produce seed in our common garden environments while some populations in the native range did produce seed. There was evidence that the evolutionary modifications to reproductive systems promote the colonising ability of invading populations (Barrett *et al.*, 2008; Castro *et al.*, 2016). Therefore, it is important to assess the evolution of latitudinal cline in both sexual and clonal reproduction related traits (Maron *et al.*, 2004; Colautti & Barrett, 2013) and the evolutionary consequence resulting from allocation trade-offs between asexual and sexual reproduction following exotic plant invasions (Barrett *et al.*, 2008; Vallejo-Marín *et al.*, 2010). In addition, the use of total biomass after 8 wk of vegetative growth as metric might provide biased estimates of fitness if there was a trade-off between growth and sexual reproduction (Etterson & Shaw, 2001; Colautti & Barrett, 2013; Castro *et al.*, 2016). Long-term experiments covering all life stages and combinations of testing asexual and sexual reproduction are therefore needed in future studies on the adaptive evolution of invasive plants. Additionally, a comprehensive understanding of how invasive plants respond to the altered herbivory along latitude in introduced range should take induced resistance and tolerance into consideration (Wieski & Pennings, 2014; Anstett *et al.*, 2016a).

Conclusion

Although comparisons of genetically based latitudinal clines in plant traits between native and introduced populations have been widely used to examine the rapid local adaptation of invasive plants, the clines in growth and defence traits have seldom been tested at the same time. We found strong evidence for clinal evolution in growth and defence in introduced populations of *A. philoxeroides*. The clines of some growth-related traits may have evolved in parallel to similar latitudinal abiotic gradients between native and introduced range. Nonparallel clines in growth rate and defence and their trade-off suggested that altered herbivory gradient might have resulted in nonparallel evolution of clines in growth and defence between native and introduced populations. The rapid evolution of growth- and defence-related traits and phenotypic plasticity to abiotic factors may have played an important role in the invasion of *A. philoxeroides*, and are likely to promote its invasion in the face of global change.


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Author contributions


YY, ML, YP, XP conceived and designed the research; YY, ML, YP, HH, XP, AS, YH and ZZ performed the experiment and collected the data; YY analysed the data and led the writing, with major inputs from XP and BL, and further inputs from all other authors.

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Data availability

The data that supports the findings of this study are openly available in the Figshare digital repository: <https://doi.org/10.6084/m9.figshare.13448261.v1>.

References

- Alexander HM, Price S, Houser R, Finch D, Tourtellot M. 2007. Is there reduction in disease and pre-dispersal seed predation at the border of a host plant's range? Field and herbarium studies of *Carex blanda*. *Journal of Ecology* 95: 446–457.
- Alexander JM, van Kleunen M, Ghezzi R, Edwards PJ. 2012. Different genetic clines in response to temperature across the native and introduced ranges of a global plant invader. *Journal of Ecology* 100: 771–781.
- Anstett DN, Chen W, Johnson MTJ. 2016a. Latitudinal gradients in induced and constitutive resistance against herbivores. *Journal of Chemical Ecology* 42: 772–781.
- Anstett DN, Naujokaitis-Lewis I, Johnson MTJ. 2014. Latitudinal gradients in herbivory on *Oenothera biennis* vary according to herbivore guild and specialization. *Ecology* 95: 2915–2923.
- Anstett DN, Nunes KA, Baskett C, Kotanen PM. 2016b. Sources of controversy surrounding latitudinal patterns in herbivory and defense. *Trends in Ecology & Evolution* 31: 789–802.
- Barrett SCH, Colautti RI, Eckert CG. 2008. Plant reproductive systems and evolution during biological invasion. *Molecular Ecology* 17: 373–383.
- Bezemer TM, Harvey JA, Cronin JT. 2014. Response of native insect communities to invasive plants. *Annual Review of Entomology* 59: 119–141.
- Bhattarai GP, Meyerson LA, Anderson J, Cummings D, Allen WJ, Cronin JT. 2017. Biogeography of a plant invasion: genetic variation and plasticity in latitudinal clines for traits related to herbivory. *Ecological Monographs* 87: 57–75.
- Blossey B, Notzold R. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* 83: 887–889.

- van Boheemen LA, Atwater DZ, Hodgins KA. 2019a. Rapid and repeated local adaptation to climate in an invasive plant. *New Phytologist* 222: 614–627.
- van Boheemen LA, Bou-Assi S, Uesugi A, Hodgins KA. 2019b. Rapid growth and defence evolution following multiple introductions. *Ecology and Evolution* 9: 7942–7956.
- Bosssdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144: 1–11.
- Castro S, Castro M, Ferrero V, Costa J, Tavares D, Navarro L, Loureiro J. 2016. Invasion fosters change: independent evolutionary shifts in reproductive traits after *Oxalis pes-caprae* L. introduction. *Frontiers in Plant Science* 7: e874.
- Chapin FS, Autumn K, Pugnaire F. 1993. Evolution of suites of traits in response to environmental stress. *American Naturalist* 142: S78–S92.
- Colautti RI, Barrett SCH. 2013. Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science* 342: 364–366.
- Colautti RI, Lau JA. 2015. Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Molecular Ecology* 24: 1999–2017.
- Colautti RI, Maron JL, Barrett SCH. 2009. Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. *Evolutionary Applications* 2: 187–199.
- Coley P. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* 53: 209–233.
- Cronin JT, Bhattarai GP, Allen WJ, Meyerson LA. 2015. Biogeography of a plant invasion: plant–herbivore interactions. *Ecology* 96: 1115–1127.
- R Core Team. 2018. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Daday H. 1954. Gene frequencies in wild populations of *Trifolium repens* L. Distribution by latitude. *Heredity* 8: 61–78.
- Daday H. 1958. Gene frequencies in wild populations of *Trifolium repens* L. III. World distribution. *Heredity* 12: 169–184.
- Daday H. 1965. Gene frequencies in wild populations of *Trifolium repens* L. IV. mechanism of natural selection. *Heredity* 20: 355–365.
- Davidson AM, Jennions M, Nicotra AB. 2011. Do invasive species show higher phenotypic plasticity than native species and if so, is it adaptive? A meta-analysis. *Ecology Letters* 14: 419–431.
- Dlugosch KM, Parker IM. 2008. Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters* 11: 701–709.
- Etterson JR, Delf DE, Craig TP, Ando Y, Ohgushi T. 2008. Parallel patterns of clinal variation in *Solidago altissima* in its native range in central USA and its invasive range in Japan. *Botany-Botanique* 86: 91–97.
- Etterson JR, Shaw RG. 2001. Constraint to adaptive evolution in response to global warming. *Science* 294: 151–154.
- Faizal A, Geelen D. 2013. Saponins and their role in biological processes in plants. *Phytochemistry Reviews* 12: 877–893.
- Forister ML, Novotny V, Panorska AK, Baje L, Basset Y, Butterill PT, Cizek L, Coley PD, Dem F, Diniz IR *et al.* 2015. The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences, USA* 112: 442–447.
- Fox J, Weisberg S. 2019. *An R companion to applied regression*. Thousand Oaks, CA, USA: Sage.
- García D, Zamora R, Gomez JM, Jordano P, Hódar JA. 2000. Geographical variation in seed production, predation and abortion in *Juniperus communis* throughout its range in Europe. *Journal of Ecology* 88: 436–446.
- Geng Y, van Klinken RD, Sosa A, Li B, Chen J, Xu C. 2016. The relative importance of genetic diversity and phenotypic plasticity in determining invasion success of a clonal weed in the USA and China. *Frontiers in Plant Science* 7: 213.
- Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287: 308–309.
- Janzen D. 1967. Why mountain passes are higher in tropics. *American Naturalist* 101: 233–249.
- Johnson MTJ, Rasmann S. 2011. The latitudinal herbivory–defence hypothesis takes a detour on the map. *New Phytologist* 191: 589–592.
- van Kleunen M, Bosssdorf O, Dawson W. 2018. The ecology and evolution of alien plants. *Annual Review of Ecology, Evolution, and Systematics* 49: 25–47.
- van Kleunen M, Fischer M. 2008. Adaptive rather than non-adaptive evolution of *Mimulus guttatus* in its invasive range. *Basic and Applied Ecology* 9: 213–223.
- Lande R, Arnold S. 1983. The measurement of selection on correlated characters. *Evolution* 37: 1210–1226.
- Latimer AM, Jacobs BS, Gianoli E, Heger T, Salgado-Luarte C. 2019. Parallel functional differentiation of an invasive annual plant on two continents. *AoB Plants* 11: plz010.
- Lenth R. 2019. *Emmeans: estimated marginal means, aka least-squares means*. [WWW document] URL <https://CRAN.R-project.org/package=emmeans>.
- Li J, Du L, Guan W, Yu F-H, van Kleunen M. 2016. Latitudinal and longitudinal clines of phenotypic plasticity in the invasive herb *Solidago canadensis* in China. *Oecologia* 182: 755–764.
- Li R. 2018. *Genetic relationship and geographic source of invasive species Alternanthera philoxeroides in China and Japan*. MA thesis, Yunnan University, Yunnan, China.
- Liu M, Pan X, Zhang Z, van Kleunen M, Li B. 2020a. Testing the shifting defense hypothesis for constitutive and induced resistance and tolerance. *Journal of Pest Science* 93: 355–364.
- Liu M, Zhou F, Pan X, Zhang Z, Traw MB, Li B. 2018. Specificity of herbivore-induced responses in an invasive species, *Alternanthera philoxeroides* (alligator weed). *Ecology and Evolution* 8: 59–70.
- Liu W, Zhang Y, Chen X, Maung-Douglass K, Strong DR, Pennings SC. 2020b. Contrasting plant adaptation strategies to latitude in the native and invasive range of *Spartina alterniflora*. *New Phytologist* 226: 623–634.
- Lu X, Siemann E, Shao X, Wei H, Ding J. 2013. Climate warming affects biological invasions by shifting interactions of plants and herbivores. *Global Change Biology* 19: 2339–2347.
- Luo X, Xu X, Zheng Y, Guo H, Hu S. 2019. The role of phenotypic plasticity and rapid adaptation in determining invasion success of *Plantago virginica*. *Biological Invasions* 21: 2679–2692.
- Maron JL, Elmendorf SC, Vila M. 2007. Contrasting plant physiological adaptation to climate in the native and introduced range of *Hypericum perforatum*. *Evolution* 61: 1912–1924.
- Maron JL, Vila M, Bommarco R, Elmendorf S, Beardsley P. 2004. Rapid evolution of an invasive plant. *Ecological Monographs* 74: 261–280.
- Moles AT, Bonser SP, Poore AGB, Wallis IR, Foley WJ. 2011. Assessing the evidence for latitudinal gradients in plant defence and herbivory. *Functional Ecology* 25: 380–388.
- Molina-Montenegro MA, Naya DE. 2012. Latitudinal patterns in phenotypic plasticity and fitness-related traits: assessing the climatic variability hypothesis (CVH) with an invasive plant species. *PLoS ONE* 7: e47620.
- Muller-Scharer H, Schaffner U, Steinger T. 2004. Evolution in invasive plants: implications for biological control. *Trends in Ecology & Evolution* 19: 417–422.
- Neuffer B, Hurka H. 1986. Variation of growth form parameters in *Capsella* (Cruciferae). *Plant Systematics & Evolution* 153: 265–279.
- Pan XY, Geng YP, Sosa A, Zhang WJ, Li B. 2007. Invasive *Alternanthera philoxeroides*: biology, ecology and management. *Acta Phytotaxonomica Sinica* 45: 884–900.
- Richards CL, Bosssdorf O, Muth NZ, Gurevitch J, Pigliucci M. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* 9: 981–993.
- Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology Evolution and Systematics* 40: 245–269.
- Sosa A, Julien M, Cordero H. 2004. New research on *Alternanthera philoxeroides* (alligator weed) in its South American native range. In: Proceedings of the XI international symposium on biological control of weeds, 180–185.
- Stamp N. 2003. Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology* 78: 23–55.
- Stevens G. 1989. The latitudinal gradient in geographical range - how so many species coexist in the tropics. *American Naturalist* 133: 240–256.
- Turner KG, Hufbauer RA, Rieseberg LH. 2014. Rapid evolution of an invasive weed. *New Phytologist* 202: 309–321.
- Valladares F, Sanchez-Gomez D, Zavala MA. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* 94: 1103–1116.

- Vallejo-Marín M, Dorken ME, Barrett SCH. 2010. The ecological and evolutionary consequences of clonality for plant mating. *Annual Review of Ecology, Evolution, and Systematics* 41: 193–213.
- Wang AO, Jiang XX, Zhang QQ, Zhou J, Li HL, Luo FL, Zhang MX, Yu FH. 2015. Nitrogen addition increases intraspecific competition in the invasive wetland plant *Alternanthera philoxeroides*, but not in its native congener *Alternanthera sessilis*. *Plant Species Biology* 30: 176–183.
- Wang YJ, Mueller-Schaerer H, van Kleunen M, Cai AM, Zhang P, Yan R, Dong BC, Yu FH. 2017. Invasive alien plants benefit more from clonal integration in heterogeneous environments than natives. *New Phytologist* 216: 1072–1078.
- Wieski K, Pennings S. 2014. Latitudinal variation in resistance and tolerance to herbivory of a salt marsh shrub. *Ecography* 37: 763–769.
- Williams JL, Auge H, Maron JL. 2008. Different gardens, different results: native and introduced populations exhibit contrasting phenotypes across common gardens. *Oecologia* 157: 239–248.
- Woods EC, Hastings AP, Turley NE, Heard SB, Agrawal AA. 2012. Adaptive geographical clines in the growth and defense of a native plant. *Ecological Monographs* 82: 149–168.
- Xu CY, Zhang WJ, Fu CZ, Lu BR. 2003. Genetic diversity of alligator weed in China by RAPD analysis. *Biodiversity and Conservation* 12: 637–645.
- Zhou F, Zhang Z, Liu M, Pan X. 2017. Effects of nutrient levels on defense against specialist insects in an invasive alligator weed. *Biodiversity Science* 25: 1276–1284.

Supporting Information

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Fig. S1 Effects of origin, experiment site and soil N on plant traits of *Alternanthera philoxeroides*.

Table S1 Sampling locations of *Alternanthera philoxeroides*.

Table S2 The average temperature monthly, relative humidity monthly and duration of sunshine monthly in Yantai (July–September) and Guangzhou (June–August) during experiments (data from <http://www.nmic.cn/>).

Table S3 Effects of elongation rate on trichome density and triterpenoid saponins concentration in native and introduced *Alternanthera philoxeroides* under two soil N treatments (high N, N+; low N, N–) in two gardens (Guangzhou: GZ; Yantai: YT).

Table S4 Selection on elongation rate, trichome density and triterpenoid saponins concentration in native and introduced *Alternanthera philoxeroides* under two soil N treatments (high N, N+; low N, N–) in two gardens (Guangzhou; Yantai).

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