Journal of
Plant Ecology

Research Article

Clonal plasticity and trait stability facilitate knotweed invasion in Europe

Pei-Pei Cao[1], Wan-Dong Yin[2], Jing-Wen Bi[1], Tian-Tian Lin[3], Sheng-Yu Wang[1], Hang Zhou[2], Zhi-Yong Liao[4], Lei Zhang[1], Madalin Parepa[5], Rui-Ting Ju[1], Jian-Qing Ding[2], Ming Nie[1], Oliver Bossdorf[5], Christina L. Richards[5,6], Ji-Hua Wu[1,7] and Bo Li[1,3,*]

[1]Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, National Observations and Research Station for Wetland Ecosystems of the Yangtze Estuary, Institute of Biodiversity Science and Institute of Eco-Chongming, School of Life Sciences, Fudan University, Shanghai 200438, China, [2]State Key Laboratory of Crop Stress Adaptation and Improvement, School of Life Sciences, Henan University, Kaifeng 475004, China, [3]Ministry of Education Key Laboratory for Transboundary Ecosecurity of Southwest China, Yunnan Key Laboratory of Plant Reproductive Adaptation and Evolutionary Ecology and Centre for Invasion Biology, Institute of Biodiversity, School of Ecology and Environmental Science, Yunnan University, Kunming 650504, China, [4]CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China, [5]Plant Evolutionary Ecology, Institute of Evolution and Ecology, University of Tübingen, Tübingen 72076, Germany, [6]Department of Integrative Biology, University of South Florida, Tampa, FL 33620, USA, [7]State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Ecology, Lanzhou University, Lanzhou 730000, China

*Corresponding author. E-mail: bool@fudan.edu.cn

Handling Editor: Shaopeng Li

Received: 12 June 2024, **First Decision:** 22 June 2024, **Accepted:** 1 July 2024, **Online Publication:** 30 July 2024

Abstract

Invasive plant species pose significant ecological and economic threats due to their establishment and dominance in non-native ranges. Previous studies have yielded mixed results regarding the plants' adaptive mechanisms for thriving in new environments, and particularly, little is known about how the phenotypic plasticity of growth and defense-related traits may facilitate plant invasion. This study addressed these uncertainties by employing the aggressive weed *Reynoutria japonica* as a study model. We examined the differences in growth, defense-related traits and biomass allocation between *R. japonica* populations from native and introduced ranges grown in two common gardens with distinct climate conditions. Our results demonstrated that while the introduced populations did not exhibit increases in height and total dry mass, nor reductions in leaf defense levels, their investment in leaf production was significantly higher compared to the native populations. Additionally, introduced populations displayed greater phenotypic plasticity in clonal ramet but less phenotypic plasticity in biomass production than native populations across varying environments. These findings highlight the roles of phenotypic plasticity and specific trait adaptations, such as clonality, in the successful invasion of *R. japonica*. This study has important implications for managing invasive plant species under changing environmental conditions.

Keywords: plant invasion, common garden, plant defense, biomass allocation, phenotypic plasticity, *Reynoutria japonica*

JOURNAL OF PLANT ECOLOGY | https://doi.org/10.1093/jpe/rtae067

克隆可塑性和性状稳定性促进虎杖入侵欧洲

摘要：入侵植物因其在非本地范围的建立和主导地位而对生态和经济构成了重大威胁。以往的研究在植物适应新环境的机制方面得出了不同的结论，特别是，学术界对于生长和防御相关性状的表型可塑性如何促进植物入侵，目前仍知之甚少。为此，本研究以入侵植物虎杖(*Reynoutria japonica*)为研究对象，比较了在两个气候条件不同的同质园中原产地和引入地虎杖种群在生长、防御相关性状和生物量分配方面的差异。研究结果表明，与原产地种群相比，尽管引入地种群的株高和总生物量没有增加，叶片防御水平也没有降低，但其在叶片生产上的投资显著更高。此外，与不同环境中的原产地种群相比，引入地种群在克隆分株方面表现出更大的表型可塑性，但在生物量生产方面的表型可塑性较小。这些发现强调了表型可塑性和特定性状的适应性，如克隆性在虎杖成功入侵中的作用。相关研究结果对于环境不断变化下入侵植物物种的管理具有重要意义。

关键词：植物入侵，同质园，植物防御，生物量分配，表型可塑性，虎杖(*Reynoutria japonica*)

INTRODUCTION

Invasive plant species encounter new biotic and abiotic environments when transitioning from native to the introduced ranges (Cronin *et al.* 2015; Lin *et al.* 2019). These environments may filter out heritable variations that affect fitness in introduced populations through natural selection. In particular, significant differences in the biotic environment, such as herbivory, exist between the native and introduced ranges, which more prominently impact plant traits (Xu *et al.* 2021). The enemy release hypothesis (ERH) suggests that plants can escape downregulation by natural enemies and experience lower herbivory in introduced ranges (Keane and Crawley 2002). Thus, invasive plants can undergo a series of adaptive evolutions, including reduced defense allocation, accelerated growth rates, and increased reproductive allocation (evolution of increased competitive ability, EICA, Blossey and Notzold 1995). Although many studies have compared differences in the growth and defense traits of native and introduced populations of alien plants, support for these hypotheses remains inconsistent (Callaway *et al.* 2022; Felker-Quinn *et al.* 2013).

On the other hand, the experimental sites may significantly influence variations in invasive plant traits across ranges. A few studies employing multiple common gardens (e.g., Qin *et al.* 2013; Yang *et al.* 2021) have demonstrated that differences in plant traits among ranges can vary depending on the experimental sites. One important reason is phenotypic plasticity, the ability of a single genotype to express different physiological or morphological phenotypes in varying environments, representing the adaptive ability in response to various environmental factors (Bradshaw 1965; Schlichting 1986). Theoretical studies have indicated that phenotypic plasticity is genetically based and is heritable, and it can be favored if it contributes to fitness costs in novel environments during the invasion process (Richards *et al.* 2006). Meta-analyses have demonstrated that invasive plants are more plastic than native plants (Bossdorf *et al.* 2005; Davidson *et al.* 2011). However, studies on phenotypic plasticity in plant growth and defense traits remain scarce, and existing studies suggest that introduced populations may evolve higher phenotypic plasticity in growth and defense traits than native conspecifics (Bhattarai *et al.* 2017; Yang *et al.* 2021). Future climate change bears similarities to the new environmental conditions encountered by plants when they invade new habitats, and related research is also important for predicting how invasive and non-invasive species respond to global change (Davidson *et al.* 2011).

Many studies have compared plant trait differences between native and introduced populations using common garden experiments (Callaway *et al.* 2022; Felker-Quinn *et al.* 2013). However, only a few studies have fully tested the EICA hypothesis by addressing growth and defense in the same species (Rotter and Holeski 2018). Moreover, previous studies have primarily focused on sexually reproducing species and used their seeds as experiment materials, limiting our understanding of the invasions by asexually cloned species (Agrawal *et al.* 2015; Li *et al.* 2020). Such studies using seeds fail to rule out the possibility that the environments where mother plants grow (i.e., maternal effects) influence observations and compromise the generalizability of the research results due to variations in reproduction methods

(Gruntman *et al.* 2017). Dispersal through vegetative propagation via rhizomes or their fragments is also proposed as an important mechanism that allows clonal invasive plants to colonize habitats that are spatially heterogeneous at a fine scale, e.g., *Phragmites australis* (Bhattarai *et al.* 2017) and *Alternanthera philoxeroides* (Geng *et al.* 2016). The significant role of clonality in promoting the invasive spread of *Helianthus tuberosus* has been confirmed (Bock *et al.* 2018). Still, its impact on the invasion process of plants dominated by asexual reproduction remains to be explored. Additionally, the geographical location of the common garden is crucial, as it determines the environmental factors, such as climate, that greatly affect experimental results and their interpretation (Moloney *et al.* 2009). Furthermore, replicated common garden experiments enable the detection of genotype-by-environment (G × E) interaction effects, and evidence for G × E effects has been found in most studies using this experimental design (Colautti *et al.* 2009), which is essential for testing the roles of evolutionary changes and plasticity in plant invasions (Yang *et al.* 2021).

Therefore, in this study, we employed an aggressive clonal plant species, *Reynoutria japonica* (Japanese knotweed), as a study model to investigate the differences in growth, defense traits, and plasticity of *R. japonica* populations from native (China) and introduced (Europe) ranges grown in two geographically distinct common gardens. We specifically asked the following questions: (1) do introduced populations exhibit superior growth performance and reduced defense levels compared to the native population, and (2) do introduced populations display higher phenotypic plasticity and specific trait adaptations than native populations? This study aimed to provide a scientific basis for understanding the phenotypic plasticity of growth and defense in *R. japonica* during its invasion, and the results offer insights into the management and control of invasive clonal plant species.

MATERIALS AND METHODS

Study material

Reynoutria japonica, native to East Asia, including China, Japan, and Korea, often appears as a pioneer species on volcanic slopes and has widely invaded Europe and North America (Beerling *et al.* 1994; Del Tredici 2017). After introduction, it spreads rapidly through rhizomes, especially along river banks or roadsides, forming dense stands that often extend over hundreds of square meters (Bímová *et al.* 2004). Its rapid expansion and ecological niche occupation strongly exclude native species and cause serious damage to native ecosystems and economic development (Lavoie 2017). *Reynoutria japonica* is considered a major environmental threat in Europe and North America. It is listed by the IUCN as one of the world's 100 most serious invasive alien species (Lowe *et al.* 2000). The species' expansion provides an excellent opportunity to explore the underlying mechanisms behind biological invasions.

Previous studies have shown that the introduced populations of *R. japonica* in Europe consist of a single female genotype (Hollingsworth and Bailey 2000; Zhang *et al.* 2016). This maintenance of high fitness, even at a very low level of genetic variation, suggests that introduced European populations possess broad environmental tolerance (Schlichting 1986). Field biogeographic comparisons have shown that *R. japonica* in France (introduced) faced fewer herbivorous natural enemies, suffered from less herbivory damage, and performed better than those in Japan (native) (Maurel *et al.* 2013). The compound composition of *R. japonica* extracts differed between native China and introduced Europe (Fan *et al.* 2009). Nutrient addition experiments indicated that the superior ability to exploit variable environments could be a key mechanism for the successful invasion of *R. japonica* (Parepa *et al.* 2013). However, research on the differences in the plant traits and environmental tolerance of native or introduced *R. japonica* remains limited (Rouifed *et al.* 2018).

Common garden design

The plant materials used in this study were collected from a cross-latitudinal survey of *R. japonica* across the native range of China and the introduced ranges of Europe (see Irimia *et al.* 2023 for more details).

We established two common gardens at Fudan University in Shanghai (31.33° N, 121.50° E) and Henan University in Kaifeng (34.82° N, 114.30° E). The two experimental sites differed in climatic conditions (Supplementary Table S1 and Fig. S1). We used rhizomes of 10 populations in each garden, each from the native range in China and the introduced range in Europe, with four individuals per population (Fig. 1; Supplementary Table S2). Individuals in these populations were all octoploid and were evenly distributed across the latitudes of the two ranges (Fig. 1; Supplementary Table S2). In total, we grew 2 ranges × 10 populations × 4

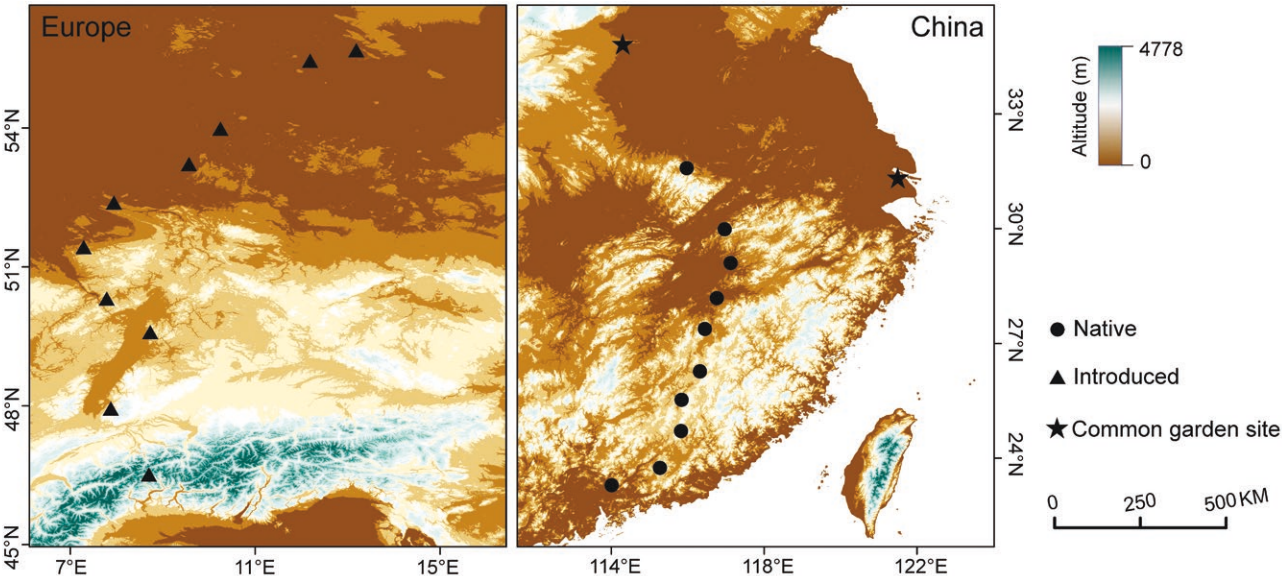

Figure 1: The locations of *Reynoutria japonica* source populations used in this study and the sites of common garden experiments.

individual plants = 80 plants within each common garden. On 11 March 2022, we treated all rhizomes with fungicide and planted them into 20-L plastic pots filled with 3.2 kg of potting soil (Pindstrup Mosebrug A/S, Denmark). All pots were on a flat clearing ground and covered with artificial grass mats. The distance between pots was maintained at least 90 cm to avoid aboveground interference (see Cao *et al.* 2024 for more planting details). In October 2022, we removed all aboveground parts when most plants showed leaf senescence. In the spring of 2023, plants in each pot sprouted independently, and by March, all individuals in the Shanghai and Kaifeng common gardens had successfully germinated.

In the second year, at the beginning of the growing season (early April), mid-June and late June, 10 g of Osmocote fertilizer (Everris International B.V., Heerlen, Netherlands, N:P:K = 16:8:12), 500 mL of diluted (3:400) water-soluble fertilizer (Miracle-Gro All Purpose Plant Food, Scotts Miracle-Gro Products, Inc., Marysville, OH, USA, N:P:K = 30:14:16) and 3 g of Stanley compound fertilizer (Stanley Agriculture Group Co., Ltd, Shandong, China, N:P:K = 17:17:17) were added to each pot, respectively. The plants were checked daily and watered as needed when the potting soil became dry during the experiment. We placed a tray under each pot to prevent the loss of water and nutrients. All plants were grown in the two common gardens for 7 months.

Measurements

Plant growth-related traits

At the end of the growth period, the length of the tallest shoot in each pot was measured from the soil surface (±0.1 cm) and as the plant height. Additionally, to measure the asexual cloning ability of *R. japonica*, we counted the number of ramets higher than 10 cm (taller ramets) and those lower than 10 cm (shorter ramets) in each pot (Wang *et al.*, unpublished data). All plants were harvested and separated into stem, leaf and root, dried at 75 °C for at least 72 h to a constant weight, and weighed (±0.01 g). Leaf dry mass for each plant was determined by summing the dry weight of the sampled leaves from the midterm and those at harvest; leaf dry mass and stem dry mass were combined to calculate the shoot dry mass. To assess biomass allocation patterns, we calculated the ratios of leaf dry mass to stem dry mass (LSR) and root dry mass to shoot dry mass (RSR) for all plants.

Leaf structural traits and chemistry

We quantified four leaf functional traits: leaf thickness, toughness, C:N ratio and flavonoids content, which are putatively associated with leaf resistance against herbivores (Callaway *et al.* 2022; Lin *et al.* 2015). The top three fully developed leaves on the tallest shoot were collected to determine plant leaf traits. Four to five leaves were collected to meet testing requirements when individuals had smaller leaves. Leaf thickness and toughness for each plant were determined by calculating the mean values of all sampled leaves for each individual. Specifically, leaf thickness was measured using a digital micrometer (Digimatic Outside Micrometer, Mitutoyo, Japan), and leaf toughness was assessed using a penetrometer (FA10, SAUTER, Balingen, Germany). Subsequently, all leaves were dried at 60 °C for at least 72 h to prepare for chemical determinations.

Leaf total carbon (C), nitrogen (N) and flavonoids content were measured in the dried leaves after the samples were ground to the required particle size using a ball mill (MM400, Retsch, Germany). Total carbon (C) and nitrogen (N) were quantified using an organic elemental analyzer (FlashSmart Elemental Analyzer, Thermo-Fisher Scientific, USA) through thermal combustion and TCD/IR detection of CO_2/N_2. At the same time, leaf flavonoids content was measured using reagent kits (Suzhou Comin Biotechnology Co., Ltd, Suzhou, China).

Data analysis

All analyses were performed using R version 4.2.1 (R Core Team 2022). We used linear mixed models to examine the effects of origin, garden and their interactions on plant traits within each common garden. These models treated the origin (China, Europe), garden (Shanghai, Kaifeng) and their interactions as fixed and population as random effects. The significance of the fixed effect was assessed using the Wald chi-squared test within the *car* package (Fox and Weisberg 2018). For traits displaying a significant origin effect ($P < 0.05$), a Tukey *post hoc* test was conducted using the *emmeans* () and *pairs* () functions within the *emmeans* package (Lenth 2018).

To test whether leaf traits of populations from native and introduced ranges differed in phenotypic plasticity, we compared the differences in the slopes of reaction's norms to garden conditions (experiment sites) for each plant trait across ranges (Valladares *et al.* 2006; Yang *et al.* 2021). We used mixed-effects models, treating origin, garden and their interaction as fixed factors, and the population was treated as a random effect. Specifically, we employed Tukey HSD *post hoc* tests to examine differences in trait mean values under various garden treatments in the mixed model, particularly when native and introduced populations exhibited differing responses to garden treatment (significant interactions involving origin × garden; Yang *et al.* 2021).

To explore whether there was a quantitative trade-off between plant dry mass and leaf defense-related traits in native and introduced populations of *R. japonica*, we performed Spearman's rank correlation analyses to calculate the correlation coefficient between leaf trait mean and total dry mass mean of each population within each garden using the *Hmisc* package (Hauke and Kossowski 2011). Data sets were separately analyzed for each range (native, introduced) at each experimental site (Shanghai, Kaifeng).

RESULTS

Growth performance

Origin of populations (ranges) had significant effects on plant height, number of ramets, stem dry mass and LSR, while experimental sites had a substantial impact on height, leaf-, stem-, root- and total dry mass, as well as LSR and RSR (Fig. 2; Table 1). Additionally, the interaction between origin and garden significantly affected the number of ramets, stem-, root- and total dry mass and LSR (Fig. 2; Table 1).

In Shanghai and Kaifeng common gardens, the plant height of native populations was 21% and 32% taller compared to introduced populations (Fig. 2a; Table 1). While the number of taller ramets in the introduced populations was respectively 49% and 325% higher than that in the native populations (Fig. 2b). The number of shorter ramets in the introduced populations was respectively 11.7 and 13.0 times higher than that of the native populations (Fig. 2c). The native populations exhibited notably higher stem-, root- and total dry mass compared to the introduced populations in the Shanghai common garden (Fig. 2). However, only the stem dry mass significantly differed in the Kaifeng common garden (Fig. 2e). The LSR of the introduced populations was respectively 69% and 26% higher than that of the native populations in Shanghai and Kaifeng gardens (Fig. 2h). However, no significant differences were observed in leaf dry mass and RSR between native and introduced populations in both common gardens (Fig. 2d and i).

Leaf defense-related traits

Concerning leaf defense-related traits, the origin of populations alone affected leaf thickness, and garden affected leaf C:N ratio and flavonoids content (Fig. 3; Table 1). Both origin and garden treatment, but not their interaction, significantly affected leaf toughness (Fig. 3; Table 1). Specifically, compared with the native populations, introduced populations

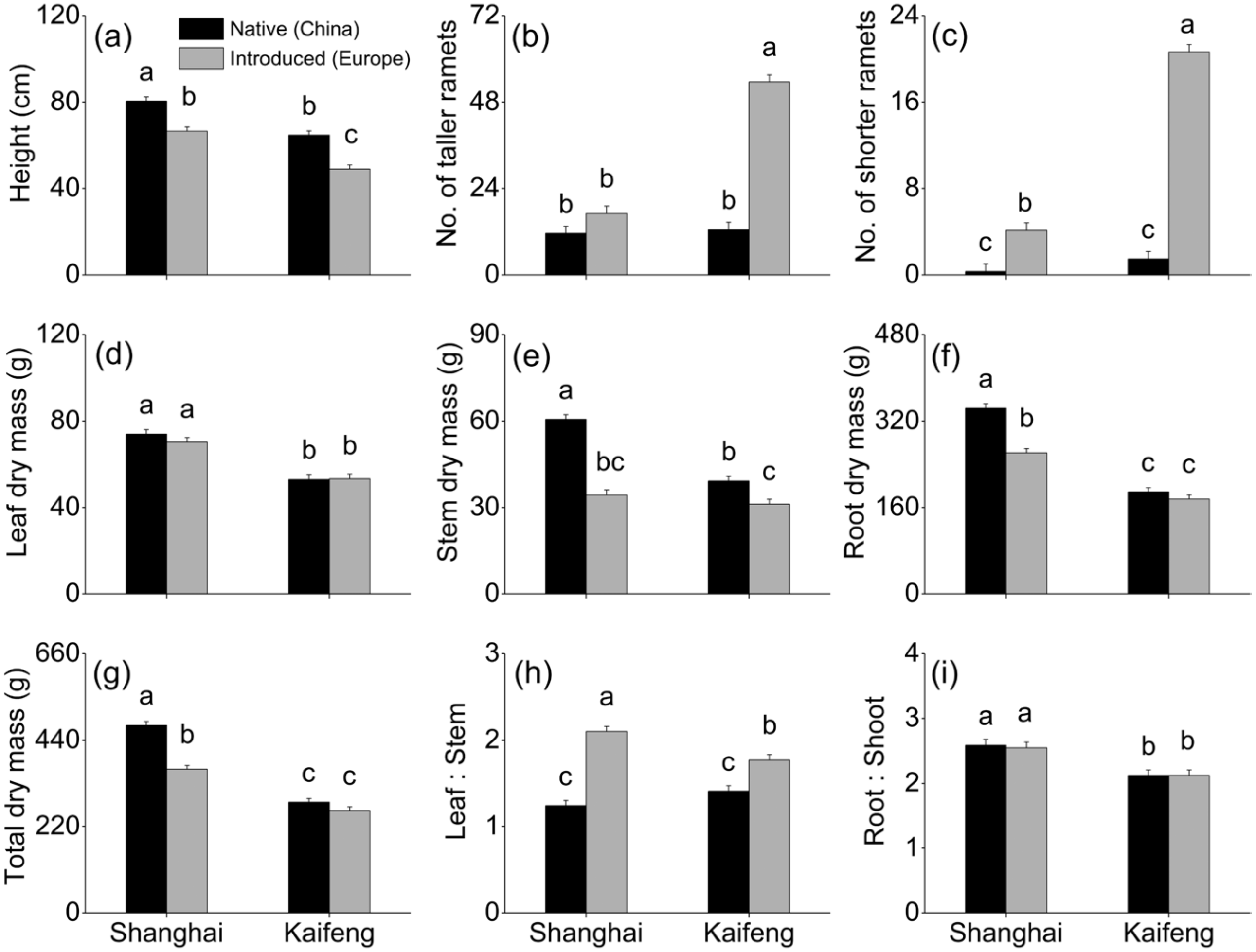

Figure 2: Growth performance of *Reynoutria japonica* from native (China) and introduced (Europe) ranges in Shanghai and Kaifeng. (a) Height, (b) number of taller ramets, (c) number of shorter ramets, (d) leaf dry mass, (e) stem dry mass, (f) root dry mass, (g) total dry mass, (h) LSR and (i) RSR. $N = 40$ for each treatment. Values are means ± SD. Different letters indicate significant differences among treatments at $P < 0.05$ based on linear mixed models followed by Tukey's HSD *post hoc* tests.

displayed 23% and 19% higher leaf thickness, with 10% and 14% higher toughness in the Shanghai and Kaifeng gardens, respectively (Fig. 3a and b). Differences in leaf C:N ratio and flavonoids content were insignificant between native and introduced populations in both common gardens (Fig. 3).

Phenotypic plasticity of plant trait

Native and introduced populations responded differently to the experimental site in terms of numbers of taller and shorter ramets, stem-, root- and total dry mass (significant O × G interactions; Table 1). For traits such as stem-, root- and total dry mass, the absolute variations were more pronounced in the native populations (Fig. 4). Conversely, the absolute increase in numbers of taller and shorter ramets was lower in the native populations than in the introduced populations when grown in Kaifeng, compared with Shanghai (Fig. 4a and b). The response of LSR in the introduced populations was the opposite, yet of approximately the same magnitude as that in the native populations (Fig. 4f).

Correlation between growth and defense-related traits

We further tested the correlations between plant growth and defense-related traits to examine the trade-offs between plant growth and defense. We found a significant positive correlation between the leaf C:N ratio and the total dry mass of the native populations in Shanghai (Table 2). However, no significant correlations were found between other leaf defense-related traits and total dry mass in the

Table 1: Effects of origin (O), garden (G) and their interactions, as well as populations, on plant traits of *Reynoutria japonica*

Variable	DF	Height	No. of taller ramets	No. of Shorter ramets	Leaf dry mass	Stem dry mass	Root dry mass	Total dry mass	LSR	RSR	Thickness	Toughness	C:N ratio	Flavonoids
		χP	χP	χP	χP	χP	χP	χP	χP	χP	χP	χP	χP	χP
Origin	1	34.23***	235.02***	402.08***	0.01	12.46***	1.71	2.66	18.27***	<0.01	79.00***	8.75**	0.45	0.02
Garden	1	111.24***	0.28	1.45	79.05***	161.67***	426.56***	544.47***	4.18*	19.77***	1.48	54.13***	24.49***	5.23*
O × G	1	0.83	136.63***	129.25***	1.46	58.57***	42.38***	58.37***	18.43 ***	0.08	2.19	0.04	1.76	0.67
Population	1	1147.46***	44.57***	4.76*	639.27***	595.71***	656.48***	924.24***	562.60**8	640.20***	4219.81***	873.89***	2136.31***	255.30***

DF = degree of freedom for each test. Statistical significance is indicated as: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

native and introduced populations in both common gardens.

DISCUSSION

Range differences in plant growth and allocation

Unlike native populations, introduced populations displayed lower plant height and dry mass (Fig. 2), which contradicted the predictions of the EICA hypothesis, which posits that introduced populations may exhibit higher growth (Blossey and Notzold 1995). Several studies have shown that plants within the introduced range often grow larger than their native counterparts (Leger and Rice 2003; Rotter *et al.* 2019), while some studies report comparable growth in native and introduced populations (Buschmann *et al.* 2005; Müller and Martens 2005). Additionally, reduced plant vigor has been observed in introduced populations of certain species (Hinz and Schwarzlaender 2004).

On the contrary, introduced populations exhibited significantly more clonal ramets than native populations (Fig. 2). The smaller plant size, coupled with an increased number of asexual clonal offshoots, can be explained by the Evolutionary Reduced Competitive Ability (ERCA) hypothesis (Bossdorf *et al.* 2004). This hypothesis posits that invasive plants reduce their energy investment in competitive traits that are costly in terms of resources when competitors in the invasive community are fewer or weaker. A reduction in competitive ability can decrease intraspecific interactions and enhance population fitness if plants in introduced populations generally have more intraspecific neighbors (Bossdorf *et al.* 2004). A study has shown that *Solidago canadensis* from introduced populations exhibit larger leaves, usually shorter than native populations in a common garden (van Kleunen and Schmid 2003). In our study, the high density of ramets of invasive knotweeds undoubtedly intensified intraspecific resource competition, resulting in shorter plants than native populations (Fig. 2). This result also suggests that the successful invasion of knotweed in Europe may rely more on the quantity of asexual clonal offshoots than their size.

Although the shoot-, root- and total dry mass of the introduced populations were lower than that of the native populations (not significant in the Kaifeng common garden), there was no significant difference in leaf dry mass between the source origins, a result of differing biomass allocation patterns (Fig. 2).

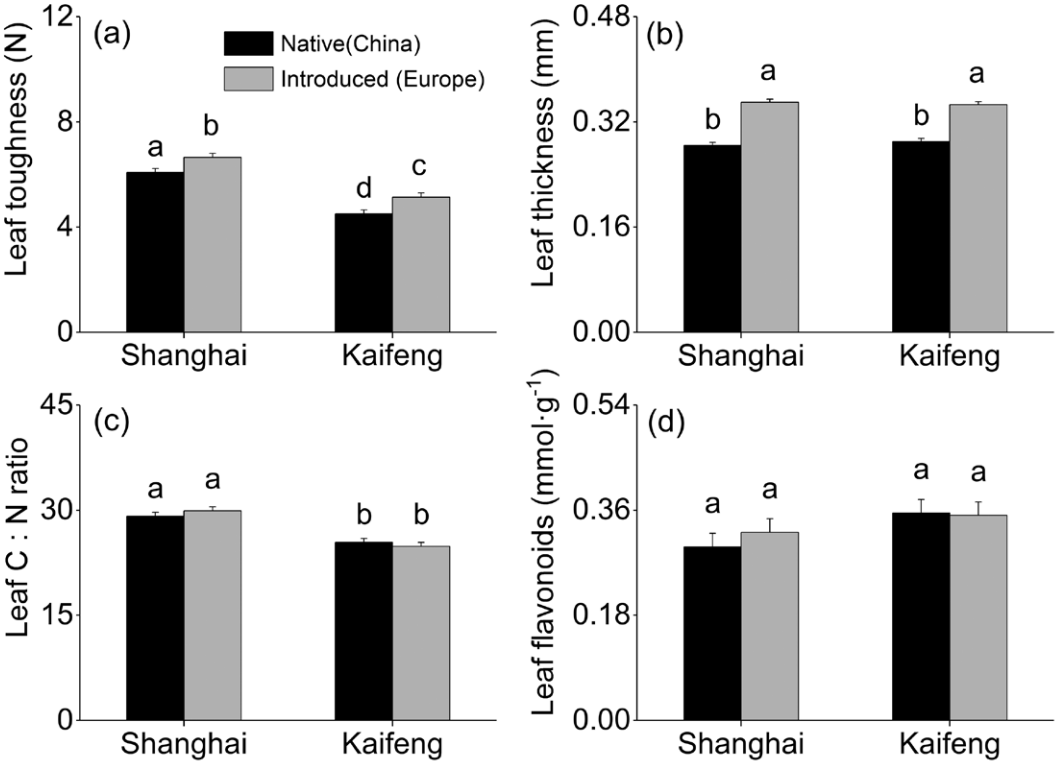

Figure 3: Leaf defense traits of *Reynoutria japonica* from native (China) and introduced (Europe) ranges in Shanghai and Kaifeng. (a) Leaf toughness, (b) leaf thickness, (c) leaf C:N ratio and (d) leaf flavonoids. $N = 40$ for each treatment. Values are means ± SD. Different letters indicate significant differences among treatments at $P < 0.05$ based on linear mixed models followed by Tukey's HSD *post hoc* tests.

Introduced populations allocated more biomass to leaves, whereas native populations allocated more to stems. The former exhibited a significantly higher leaf:stem ratio (Fig, 2), a larger specific leaf area and a higher relative chlorophyll content (Wang *et al.*, unpublished data), suggesting that introduced populations may enhance their light competitive ability by investing more energy into leaf production (Heberling and Fridley 2013; van Kleunen *et al.* 2011). Feng *et al.* (2009) have discovered that this also holds for plants from introduced populations of *Ageratina adenophora*, contributing to invasion success by favoring genotypes with high specific leaf area, photosynthetic rate and nitrogen use efficiency.

Range differences in leaf defense-related traits

We found that introduced populations exhibited higher leaf thickness and toughness, with no significant difference observed in leaf chemical defense traits (Fig. 3), which contradicted another prediction of the EICA hypothesis that introduced populations exhibit lower defense levels than native populations; however, previous studies have provided mixed support for this hypothesis (Bossdorf *et al.* 2005; Rotter and Holeski 2018). A survey of the invasive plant *Phragmites australis* has revealed that the levels of leaf defense traits of introduced populations, including leaf toughness, leaf phenolics and leaf carbon, are not significantly different from those of the native populations (Bhattarai *et al.* 2017). Other common garden experiments on invasive plants, such as studies on *Chromolaena odorata* (Liao *et al.* 2014), *Brassica nigra* (Oduor *et al.* 2011) and *Verbascum Thapsus* (Endriss *et al.* 2018), have demonstrated that populations in invaded areas exhibit similar or higher defense levels than their native populations. Rouifed *et al.* (2018) suggest that introduced populations of *R. japonica* show no differences in secondary metabolite composition, stem stiffness and leaf thickness compared to native populations. Yet, these populations exhibited significantly greater leaf toughness in common gardens. Furthermore, beyond defense against herbivores, leaf functional traits such as thickness and flavonoids content play crucial roles in other aspects, including resistance to abiotic stress like ultraviolet radiation and drought (Barton and Boege 2017; Yin *et al.* 2023)

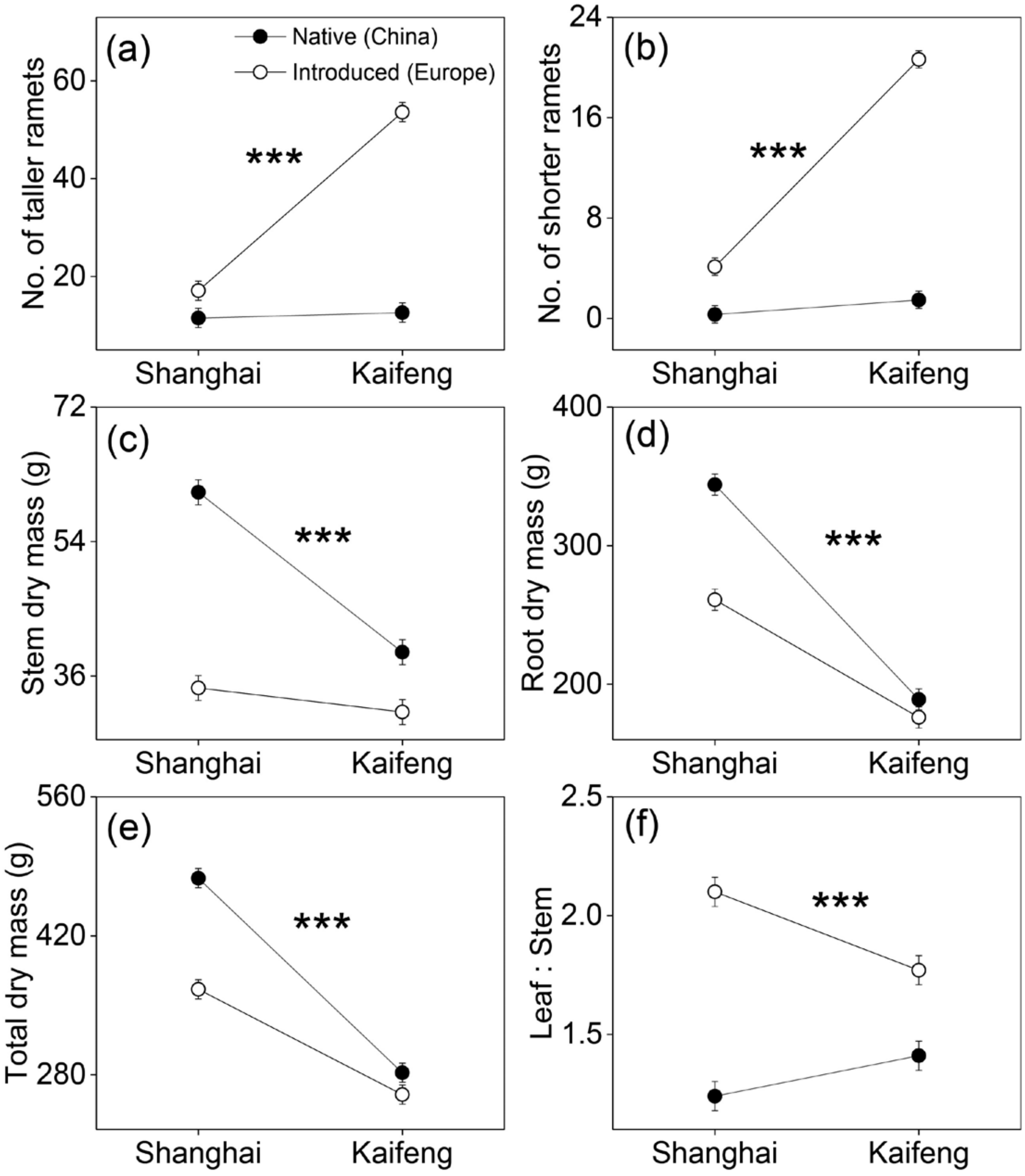

Figure 4: Interactions between origin and garden (experiment site) on plant traits that significantly affected by origin × garden in Table 1. (a) Number of taller ramets, (b) the number of shorter ramets, (c) stem dry mass, (d) root dry mass, (e) total dry mass and (f) LSR. $N = 40$ for each treatment. Points are means ± SD. The significance level of origin × garden is indicated by $^{***}P < 0.001$.

and in enhancing plant competitiveness through allelopathy (Zhang *et al.* 2021).

Growth and defense within populations should theoretically trade off against each other to optimize fitness due to physiological constraints or priorities for growth or defense (Yang *et al.* 2021). However, less than 20% of studies have found evidence of trade-offs within species (Hahn and Maron 2016). Intraspecific correlations between growth and defense can vary with resource availability, with a negative or no growth-defense trade-off in low-resource areas and a positive correlation between growth and defense in high-resource regions, respectively (Hahn *et al.* 2021). We did not find trade-offs between growth and defense-related traits in native and introduced populations, possibly due to higher resource availability in common gardens (Table 2).

Our study further found that the plant traits varied substantially in the two gardens. For instance, plants tended to be taller in Shanghai, with higher leaf-, stem-, root- and total dry mass, RSR, leaf toughness and C:N ratio, but fewer ramets (Figs 2 and 3). Inconsistency might be attributable to the differences

Table 2: Spearman's rank correlation coefficient between total dry mass and leaf defense-related traits of native and introduced *Reynoutria japonica*

		Total dry mass (Shanghai)		Total dry mass (Kaifeng)	
Variable	**Range**	***r***	***P***	***r***	***P***
Thickness	Native	−0.19	0.229	−0.29	0.069
	Introduced	−0.05	0.747	0.21	0.188
Toughness	Native	−0.21	0.191	0.02	0.905
	Introduced	−0.18	0.280	−0.19	0.240
C:N ratio	Native	**0.56**	**<0.001**	−0.22	0.182
	Introduced	−0.02	0.892	−0.18	0.266
Flavonoids	Native	−0.01	0.966	−0.17	0.306
	Introduced	0.15	0.360	0.11	0.490

Bold indicates a significant correlation ($P < 0.05$).

in abiotic and biotic factors between the two common gardens (Supplementary Table S1 and Fig. S1). The higher plant height and dry mass in the Shanghai common garden might be due to the higher humidity in this area, which more closely resembles the environmental conditions of its field habitats, such as riparian or riverside areas (Supplementary Table S2; Del Tredici 2017). Our results thus underscore the importance of utilizing multiple common gardens to test the growth and defense of invasive plants (Qin *et al.* 2013; Yang *et al.* 2021).

Range differences in phenotypic plasticity of plant traits

The evolution of increased phenotypic plasticity hypothesis postulates that alien plants have developed enhanced phenotypic plasticity in invaded areas compared to their native ranges (Bossdorf *et al.* 2005; Richards *et al.* 2006), and our findings partially corroborate this hypothesis (Fig. 4). We found that introduced populations exhibited a higher phenotypic plastic response in the number of ramets compared to native populations in two common garden environments (Fig. 4a and b). These results aligned with other studies documenting increased phenotypic plasticity in introduced populations, e.g. in *P. australis* (Bhattarai *et al.* 2017), *C. odorata* (Liao *et al.* 2020) and *Alternanthera philoxeroides* (Yang *et al.* 2021).

Previous studies have indicated that the introduced populations of *R. japonica* in Europe and North America are composed of a single female genotype (Hollingsworth and Bailey 2000; Zhang *et al.* 2016) and expanded primarily through clonal propagation of a single asexual lineage (Gaskin *et al.* 2014; Richards *et al.* 2012). Our results suggest that the successful invasion of *R. japonica* in Europe is largely attributed to rhizome growth and enormous phenotypic plasticity in clonal ramet capacity. Contrary to the number of ramets, we found that the phenotypic plasticity of stem-, root- and total dry mass of the introduced populations was significantly lower than that of the native populations (Fig. 4), meaning that the introduced populations were more adept at maintaining dry mass stability in varying environments (a jack-of-all-trades, Richards *et al.* 2006). This phenomenon may partially explain the success of invasive species in varying environments.

We used a relatively large number of populations within China (native) and Europe (introduced) and conducted common gardens at two experimental sites with different climatic conditions (Supplementary Table S1), which resulted in relatively robust results. However, the ancestor of the European populations can be traced to Nagasaki, Japan (Beerling *et al.* 1994; Zhang *et al.*, unpublished data). Consequently, future comparisons with Japanese populations can provide more insights into the mechanisms underlying the successful invasion of *R. japonica*.

CONCLUSIONS

Our study utilized 2 common gardens and analyzed 10 populations from native and introduced ranges to assess differences in growth and leaf defense traits contributing to the invasiveness of Japanese knotweed. Our results indicated that neither the height nor the dry mass of Japanese knotweed in introduced populations increased, nor did the level of leaf defense decrease compared to native populations. The biomass allocation pattern in introduced populations, particularly the investment in leaves, was significantly higher than in native populations. The greater plasticity in ramet number and reduced plasticity in dry mass, characteristic of a 'jack-of-all-trades', may underpin its successful invasion in Europe. This study offers important insights into the expansion mechanisms of *R. japonica* on a large spatial scale. It provides a clear example of how clonality promotes the successful invasion of alien plants. For invasive plants like knotweed that primarily asexually reproduce through clonal growth, control measures targeting the rhizomes may be more effective.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: The monthly maximum, minimum and average temperature, precipitation and relative humidity in Shanghai and Kaifeng during experiments (February–August; data from https://www.qweather.com/).

Table S2: Sampling locations of *Reynoutria japonica.*

Figure S1: Principal component analysis (PCA) for climate variables during the Shanghai and Kaifeng common garden experiments.

Funding

This work was supported by the National Natural Science Foundation of China (grant 31961133028), the German Federal Ministry of Education and Research (MOPGA Project 306055), the German Research Foundation (grant 431595342) and the Department of Science and Technology of Yunnan Province (grant 202405AS350011).

Acknowledgements

We thank Elodie Kugler, Rongjin Li, Kaitao Liu, Rui Min, Julia Rafalski, Eva Schloter, Weihan Zhao, Yujie Zhao, Wenchao Zhong, Zhiming Zhong and Xin Zhuang for help with field collections, and Chenxu Bao, Yujiao Ding, Jieren Jin, Haiyan Li, Chaonan Wang and Jiawei Wang with common garden experiments. We also thank the Center for Gardening and Horticulture and the Seed Bank of Tropical Plant Resources at XTBG for providing quarantine facilities and cold rooms.

Conflict of interest statement. The authors declare that they have no conflict of interest.

REFERENCES

Agrawal AA, Hastings AP, Bradburd GS, *et al.* (2015) Evolution of plant growth and defense in a continental introduction. *Am Nat* **186**:E1–E15. https://doi.org/10.1086/681622

Barton KE, Boege K (2017) Future directions in the ontogeny of plant defence: understanding the evolutionary causes and consequences. *Ecol Lett* **20**:403–411. https://doi.org/10.1111/ele.12744

Beerling DJ, Bailey JP, Conolly AP (1994) *Fallopia japonica* (Houtt.) Ronse Decraene. *J Ecol* **82**:959–979. https://doi.org/10.2307/2261459

Bhattarai GP, Meyerson LA, Anderson J, *et al.* (2017) Biogeography of a plant invasion: genetic variation and plasticity in latitudinal clines for traits related to herbivory. *Ecol Monogr* **87**:57–75. https://doi.org/10.1002/ecm.1233

Bímová K, Mandák B, Kašparová I (2004) How does *Reynoutria* invasion fit the various theories of invasibility? *J Veg Sci* **15**:495–504. https://doi.org/10.1658/1100-9233(2004)015[0495:hdrift]2.0.co;2

Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *J Ecol* **83**:887–889. https://doi.org/10.2307/2261425

Bock DG, Kantar MB, Caseys C, *et al.* (2018) Evolution of invasiveness by genetic accommodation. *Nat Ecol Evol* **2**:991–999. https://doi.org/10.1038/s41559-018-0553-z

Bossdorf O, Prati D, Auge H, *et al.* (2004) Reduced competitive ability in an invasive plant. *Ecology Lett* **7**:346–353. https://doi.org/10.1111/j.1461-0248.2004.00583.x

Bossdorf O, Auge H, Lafuma L, *et al.* (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* **144**:1–11. https://doi.org/10.1007/s00442-005-0070-z

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Adv Genet* **13**:115–155. https://doi.org/10.1016/S0065-2660(08)60048-6

Buschmann H, Edwards PJ, Dietz H (2005) Variation in growth pattern and response to slug damage among native and invasive provenances of four perennial Brassicaceae species. *J Ecol* **93**:322–334. https://doi.org/10.1111/j.1365-2745.2005.00991.x

Callaway RM, Lucero JE, Hierro JL, *et al.* (2022) The EICA is dead? Long live the EICA! *Ecol Lett* **25**:2289–2302. https://doi.org/10.1111/ele.14088

Cao PP, Liao ZY, Wang SY, *et al.* (2024). Cross-continental variation of herbivore resistance in a global plant invader. *bioRxiv*. https://doi.org/10.22541/au.172114317.76216435/v1

Colautti RI, Maron JL, Barrett SC (2009) Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. *Evol Appl* **2**:187–199. https://doi.org/10.1111/j.1752-4571.2008.00053.x

Cronin JT, Bhattarai GP, Allen WJ, *et al.* (2015) Biogeography of a plant invasion: plant-herbivore interactions. *Ecology* **96**:1115–1127. https://doi.org/10.1890/14-1091.1

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. *Ecol Lett* **14**:419–431. https://doi.org/10.1111/j.1461-0248.2011.01596.x

Del Tredici P (2017) The introduction of Japanese knotweed, *Reynoutria japonica*, into North America. *J Torrey Bot Soc* **144**:406–416. https://doi.org/10.3159/torrey-d-17-00002.1

Endriss SB, Alba C, Norton AP, *et al*. (2018) Breakdown of a geographic cline explains high performance of introduced populations of a weedy invader. *J Ecol* **106**:699–713. https://doi.org/10.1111/1365-2745.12845

Fan PH, Hay AE, Marston A, *et al*. (2009) Chemical variability of the invasive neophytes *Polygonum cuspidatum* Sieb. and Zucc. and *Polygonum sachalinensis* F. Schmidt ex Maxim. *Biochem Syst Ecol* **37**:24–34. https://doi.org/10.1016/j.bse.2008.11.018

Felker-Quinn E, Schweitzer JA, Bailey JK (2013) Meta-analysis reveals evolution in invasive plant species but little support for Evolution of Increased Competitive Ability (EICA). *Ecol Evol* **3**:739–751. https://doi.org/10.1002/ece3.488

Feng YL, Lei YB, Wang RF, *et al*. (2009) Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant. *Proc Natl Acad Sci USA* **106**:1853–1856. https://doi.org/10.1073/pnas.0808434106

Fox J, Weisberg S (2018) *An R Companion to Applied Regression*, 3rd ed. Thousand Oaks CA, USA: Sage Publications.

Gaskin JF, Schwarzländer M, Grevstad FS, *et al*. (2014) Extreme differences in population structure and genetic diversity for three invasive congeners: knotweeds in western North America. *Biol Invasions* **16**:2127–2136. https://doi.org/10.1007/s10530-014-0652-y

Geng YP, van Klinken RD, Sosa A, *et al*. (2016) The relative importance of genetic diversity and phenotypic plasticity in determining invasion success of a clonal weed in the USA and China. *Front Plant Sci* **7**:182469. https://doi.org/10.3389/fpls.2016.00213

Gruntman M, Segev U, Glauser G, *et al*. (2017) Evolution of plant defences along an invasion chronosequence: defence is lost due to enemy release–but not forever. *J Ecol* **105**:255–264. https://doi.org/10.1111/1365-2745.12660

Hahn PG, Maron JL (2016) A framework for predicting intraspecific variation in plant defense. *Trends Ecol Evol* **31**:646–656. https://doi.org/10.1016/j.tree.2016.05.007

Hahn PG, Keefover-Ring K, Nguyen LM, *et al*. (2021) Intraspecific correlations between growth and defence vary with resource availability and differ within and among populations. *Funct Ecol* **35**:2387–2396. https://doi.org/10.1111/1365-2435.13878

Hauke J, Kossowski T (2011) Comparison of values of Pearson's and Spearman's correlation coefficients on the same sets of data. *Quageo* **30**:87–93. https://doi.org/10.2478/v10117-011-0021-1

Heberling JM, Fridley JD (2013) Resource-use strategies of native and invasive plants in Eastern North American forests. *New Phytol* **200**:523–533. https://doi.org/10.1111/nph.12388

Hinz HL, Schwarzlaender M (2004) Comparing invasive plants from their native and exotic range: what can we learn for biological control? *Weed Technol* **18**:1533–1541. https://doi.org/10.1614/0890-037x(2004)018[1533:cipftn]2.0.co;2

Hollingsworth ML, Bailey JP (2000) Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese knotweed). *Bot J Linn Soc* **133**:463–472. https://doi.org/10.1111/j.1095-8339.2000.tb01589.x

Irimia RE, Zhao WH, Cao PP, *et al*. (2023) Cross-continental shifts of ecological strategy in a global plant invader. PREPRINT (Version 1) available at *Research Square*. https://doi.org/10.21203/rs.3.rs-3793794/v1

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends Ecol Evol* **17**:164–170. https://doi.org/10.1016/S0169-5347(02)02499-0

Lavoie C (2017) The impact of invasive knotweed species (*Reynoutria* spp.) on the environment: review and research perspectives. *Biol Invasions* **19**:2319–2337. https://doi.org/10.1007/s10530-017-1444-y

Leger EA, Rice KJ (2003) Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced competition. *Ecol Lett* **6**:257–264. https://doi.org/10.1046/j.1461-0248.2003.00423.x

Lenth R (2018) *Emmeans: Estimated Marginal Means, Aka Least-squares Means* (R package version 1.8.2).

Li XQ, Gao X, Siemann E, *et al*. (2020) Effects of above-and belowground herbivory of specialists and generalists on the growth and defensive chemicals of introduced and native Chinese tallow seedlings. *Plant Soil* **455**:65–78. https://doi.org/10.1007/s11104-020-04666-2

Liao ZY, Zheng YL, Lei YB, *et al*. (2014) Evolutionary increases in defense during a biological invasion. *Oecologia* **174**:1205–1214. https://doi.org/10.1007/s00442-013-2852-z

Liao ZY, Scheepens JF, Li QM, *et al*. (2020) Founder effects, post-introduction evolution and phenotypic plasticity contribute to invasion success of a genetically impoverished invader. *Oecologia* **192**:105–118. https://doi.org/10.1007/s00442-019-04566-y

Lin TT, Klinkhamer PG, Vrieling K (2015) Parallel evolution in an invasive plant: effect of herbivores on competitive ability and regrowth of *Jacobaea vulgaris*. *Ecol Lett* **18**:668–676. https://doi.org/10.1111/ele.12445

Lin TT, Klinkhamer PG, Pons TL, *et al*. (2019) Evolution of increased photosynthetic capacity and its underlying traits in invasive *Jacobaea vulgaris*. *Front Plant Sci* **10**:427842. https://doi.org/10.3389/fpls.2019.01016

Lowe S, Browne M, Boudjelas S, *et al*. (2000). *100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database* (Vol. **12**). Auckland: Invasive Species Specialist Group.

Maurel N, Fujiyoshi M, Muratet A, *et al*. (2013) Biogeographic comparisons of herbivore attack, growth and impact of Japanese knotweed between Japan and France. *J Ecol* **101**:118–127. https://doi.org/10.1111/1365-2745.12026

Moloney KA, Holzapfel C, Tielbörger K, *et al*. (2009) Rethinking the common garden in invasion research. *Perspect Plant Ecol Evol Syst* **11**:311–320. https://doi.org/10.1016/j.ppees.2009.05.002

Müller C, Martens N (2005) Testing predictions of the 'evolution of increased competitive ability' hypothesis for an invasive crucifer. *Evol Ecol* **19**:533–550. https://doi.org/10.1007/s10682-005-1022-0

Oduor AM, Lankau RA, Strauss SY, *et al*. (2011) Introduced *Brassica nigra* populations exhibit greater growth and herbivore resistance but less tolerance than native populations in the native range. *New Phytol* **191**:536–544. https://doi.org/10.1111/j.1469-8137.2011.03685.x

Parepa M, Fischer M, Bossdorf O (2013) Environmental variability promotes plant invasion. *Nat Commun* **4**:1604. https://doi.org/10.1038/ncomms2632

Qin RM, Zheng YL, Valiente-Banuet A, *et al*. (2013) The evolution of increased competitive ability, innate competitive advantages, and novel biochemical weapons act in concert for a tropical invader. *New Phytol* **197**:979–988. https://doi.org/10.1111/nph.12071

R Core Team (2022) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Richards CL, Bossdorf O, Muth NZ, *et al*. (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol Lett* **9**:981–993. https://doi.org/10.1111/j.1461-0248.2006.00950.x

Richards CL, Schrey AW, Pigliucci M (2012) Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation. *Ecol Lett* **15**:1016–1025. https://doi.org/10.1111/j.1461-0248.2012.01824.x

Rotter MC, Holeski LM (2018) A meta-analysis of the evolution of increased competitive ability hypothesis: genetic-based trait variation and herbivory resistance trade-offs. *Biol Invasions* **20**:2647–2660. https://doi.org/10.1007/s10530-018-1724-1

Rotter MC, Vallejo-Marin M, Holeski LM (2019) A test of the evolution of increased competitive ability in two invaded regions. *Evol Ecol* **33**:713–735. https://doi.org/10.1007/s10682-019-10004-5

Rouifed S, Puijalon S, Bardon C, *et al*. (2018) Comparison of defence and performance traits between one widespread clone and native populations in a major invasive plant species. *Divers Distrib* **24**:297–312. https://doi.org/10.1111/ddi.12690

Schlichting CD (1986) The evolution of phenotypic plasticity in plants. *Annu Rev Ecol Syst* **17**:667–693. https://doi.org/10.1146/annurev.es.17.110186.003315

Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *J Ecol* **94**:1103–1116. https://doi.org/10.1111/j.1365-2745.2006.01176.x

van Kleunen M, Schlaepfer DR, Glaettli M, *et al*. (2011) Preadapted for invasiveness: do species traits or their plastic response to shading differ between invasive and non-invasive plant species in their native range? *J Biogeogr* **38**:1294–1304. https://doi.org/10.1111/j.1365-2699.2011.02495.x

van Kleunen M, Schmid B (2003) No evidence for an evolutionary increased competitive ability in an invasive plant. *Ecology* **84**:2816–2823. https://doi.org/10.1890/02-0494

Xu M, Mu XD, Zhang S, *et al*. (2021) A global analysis of enemy release and its variation with latitude. *Global Ecol Biogeogr* **30**:277–288. https://doi.org/10.1111/geb.13229

Yang Y, Liu M, Pan YF, *et al*. (2021) Rapid evolution of latitudinal clines in growth and defence of an invasive weed. *New Phytol* **230**:845–856. https://doi.org/10.1111/nph.17193

Yin WD, Zhou LF, Yang KW, *et al*. (2023) Rapid evolutionary trade-offs between resistance to herbivory and tolerance to abiotic stress in an invasive plant. *Ecol Lett* **26**:942–954. https://doi.org/10.1111/ele.14221

Zhang YY, Parepa M, Fischer M, *et al*. (2016) Epigenetics of colonizing species? A study of Japanese knotweed in Central Europe. In Spencer CH, Barrett RI, Colautti KM, *et al*. (eds). *Invasion Genet*. Chichester, UK: John Wiley & Sons, Ltd, 328–340. https://doi.org/10.1002/9781119072799.ch19

Zhang ZJ, Liu YJ, Yuan L, *et al*. (2021) Effect of allelopathy on plant performance: a meta-analysis. *Ecol Lett* **24**:348–362. https://doi.org/10.1111/ele.13627