Cross-continental shifts of ecological strategy in a global plant invader

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Article

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Abstract

Plant invasions are a global problem that requires studying plants and their environmental associations across native and introduced ranges. We surveyed 150 populations of Japanese knotweed, a key invader of the temperate zone, along 2000 km transects in native China and the introduced ranges of Europe and North America. We found that larger plants and denser populations in the introduced ranges were associated with shifts in leaf economy and chemical defenses. Introduced knotweed populations had higher SLA but reduced leaf chlorophyll, lignin, C:N and leaf toughness along with altered leaf tannins, flavonoids and alkaloids. We found three distinct multivariate knotweed phenotypes primarily in the introduced ranges, and two phenotype clusters mainly in native populations. Decreased herbivore and pathogen impacts in invasive populations and changes in environmental associations indicate that enemy release and novel habitat conditions might have driven the emergence of novel ecological strategies in this global plant invader.

Introduction

Biological invasions are one of the major threats to global biodiversity \(^1,2\), but they also provide large, unplanned experiments that yield insights into complex ecological and evolutionary processes \(^3\text{--}^5\). Studying the drivers of invasion across environmental contexts can provide a fundamental understanding of species responses to novel environments \(^6\). This knowledge, in turn, can be used to manage and predict invasions, and thus mitigate future biodiversity losses. One main strategy in this context has been to link invader success in different habitats to variation in plant traits \(^7\text{--}^9\).

A variety of traits affect the growth, development, reproduction, or survival of plants under different environmental conditions \(^10,11\). Such traits can be morphological, physiological or phenological, and they often occur in clusters or ‘trait syndromes’ that are thought to reflect different ecological strategies \(^12\). One important framework for understanding trait variation is the so-called leaf economics spectrum \(^13\), which posits a main axis of trait variation from slow-growing, resource-conservative species to fast-growing species that rapidly take up resources but are less resource-efficient. The fast-growing species have short-lived leaves with, among others, high nitrogen content, photosynthetic capacity and specific leaf area (SLA), whereas the slow-growing species have longer-lived leaves with opposite characteristics. ‘Fast’ species are particularly common in nutrient-rich and disturbed habitats (a condition also associated with plant invasion \(^14\)), whereas ‘slow’ species are thought to have an advantage under strong competition, nutrient-poor or other challenging conditions.

An important dimension of plant traits that is not considered in the classic leaf economics spectrum are traits related to defenses against natural enemies \(^15\). Many plant species have morphological defenses (surface wax, trichomes, spines and thorns and higher toughness of the leaves) or produce secondary metabolites that act as toxins or reduce tissue palatability to herbivores \(^16\). Chemical defenses can be constitutively present in plant tissues, or they can be induced in response to herbivory. Further, chemical
defenses can be qualitative or quantitative. Qualitative defenses (e.g., alkaloids, glycosides or terpenes) are produced at low concentrations and are typically effective against generalist herbivores whereas quantitative defenses (e.g., lignins and tannins) are effective against a wide range of herbivores but require larger doses and high allocation costs \(^{16}\). Introduced plant populations often experience a release from specialist enemies compared to native populations of the same species, although they continue to be attacked by generalists \(^{17–19}\). This variation in herbivore pressure should select on variation in defense traits \(^{20}\), and result in shifts from specialist to generalist defenses \(^{21}\), as well as overall decreased defenses.

Besides differences between native and introduced ranges, most plant traits also harbor substantial geographic variation within ranges, which is shaped by variation in abiotic conditions, biotic interactions, and the evolutionary history of a species. Studying trait-environment relationships and their links to plant and population performance thus increases our mechanistic understanding of ecological strategies \(^{22,23}\). Conducting such studies in native versus introduced ranges may detect changes in ecological strategies during a plant invasion. However, few studies so far have undertaken this approach \(^{24,25}\), even for widespread invaders, because of the logistic challenges of sampling at many field locations throughout the native and introduced ranges \(^{22}\).

Invasive knotweeds (\textit{Reynoutria} spp., Polygonaceae) are listed among the 100 most invasive species in the world. Native to Eastern Asia, \textit{Reynoutria japonica} (Japanese knotweed) and \textit{R. sachalinensis} (Giant knotweed) were introduced to temperate Europe and North America as garden ornamentals in the nineteenth century \(^{26}\). Hybridization between the two species created the hybrid \textit{R. x bohemica} (Chrtek & Chrtková) which can have a more aggressive spread than either parent \(^{27}\). The invasive knotweeds inhabit a wide range of habitats including riverbanks, roadsides and urban habitats, and forests \(^{28–30}\). These species are powerful ecological engineers, which can reduce species richness of native communities and disrupt nutrient cycling and ecosystem stability \(^{31–34}\). The success of the invasive knotweeds has been attributed to multiple ecological and evolutionary processes including clonal regeneration \(^{35,36}\), phenotypic plasticity \(^{37,38}\), hybridization and introgression \(^{39,40}\), broad ecological amplitude \(^{41}\), production of allelopathic metabolites \(^{32,42,43}\) and a superior ability to exploit resource fluctuations \(^{44}\). However, we currently do not know whether these mechanisms play a role in the native range. There is generally limited data on how native or introduced knotweeds differ in traits, abiotic environmental tolerances, or local adaptation (but see \(^{38,45,46}\)). Moreover, factors that control knotweed performance \textit{in situ} remain poorly understood. Studies in several locations in Europe showed that knotweed abundance is linked to light intensity \(^{47}\), disturbance regimes, moisture and soil nitrogen \(^{29}\) as well as riparian land use \(^{48}\).

Here, we evaluated variation in plant performance and plant traits, and their associations with abiotic environmental factors, in natural populations of Japanese knotweed along large latitudinal gradients in its native (China) and introduced ranges (Europe and USA). We proposed the following hypotheses: (1) Compared to native populations, knotweed plants in invasive populations show increased performance
and shifts in traits. (2) Invasive populations exhibit different trait syndromes reflecting shifts in ecological strategies that support invasion. (3) Shifts in traits and strategies are associated with changes in relative importance of different putative environmental drivers.

Materials and Methods

Study species

The invasive knotweeds include three different taxa: Reynoldsia japonica (Japanese knotweed), R. sachalinensis (giant knotweed) and R. x bohemica (Bohemian knotweed). The latter is a hybrid resulting from a cross between the first two species. The parental species R. japonica and R. sachalinensis were introduced to Europe from Japan in the mid 1800's, and to the United States at the end of the 19th century. Invasive knotweeds are herbaceous perennials that spread predominantly by clonal fragmentation of rhizomes and stems but can also undergo sexual reproduction by seeds when compatible pollen sources are available. Several previous studies suggested that invasive populations of R. japonica consisted of a single female genotype in Europe and the USA (but see). Reynoldsia japonica and the hybrid are widely distributed and highly invasive, whereas the giant knotweed is less common and not usually regarded as a pest.

Field survey

We conducted a cross-latitudinal survey of 150 Japanese knotweed populations in the native range of China (23.29°N to 36.86°N) and the introduced ranges of North America (34.24°N to 44.94°N) and Europe (44.67°N to 59.94°N; see Supplementary Table 1 for details about the sampling sites) during peak growing season. We focused on R. japonica, but due to morphological similarities, we could not rule out the occasional inclusion of a similar looking hybrid in the introduced range. In China, we sampled from Xunwu in Guangdong to Zouping in Shandong between 8 July-16 August in 2020. In Europe, we sampled from Carmagnola, Italy to Uppsala, Sweden between 30 May-16 June 2019. In the United States, we sampled from Commerce, Georgia to Milford, Maine between 2-19 June 2019.

In each range, we surveyed 50 populations along a 2000 km transect (sampling approximately every 40 km; Supplementary Table 1). At each site, we measured the width and length of the population and laid a 30 m transect for sampling. We selected five knotweed stems at regular intervals along the transect at 2, 8, 14, 20 and 26 m. When a population was smaller than ~28 m in length, we reduced the distance between stems, but kept stems separated by at least one meter. For each selected stem, we measured stem density in a one square meter quadrat around the stem, the stem height and diameter. We also collected five fully developed leaves to measure leaf chlorophyll content, leaf thickness, leaf toughness, percent leaf area lost to herbivory, presence of pathogens, specific leaf area (SLA, total leaf area cm²/leaf dry mass), carbon (C), nitrogen (N), and secondary metabolites (tannins, alkaloids, lignin and flavonoids).
For the leaf chemical analyses, we ground leaf samples to the required particle size with a ball mill (MM400, Retsch, Germany). We used FlashSmart Elemental Analyzer (Thermo Scientific, Germany) via thermal combustion and TCD/IR detection of CO$_2$/N$_2$ to analyze total C and N. We analyzed leaf lignin and secondary metabolites (tannins, alkaloids and flavonoids) with reagent kits following the manufacturer’s protocol (Suzhou Comin Biotechnology Co., Ltd., Suzhou, China) with some modifications. Briefly, to measure lignin, we added 4 mg of dry samples to a mixture of acetic acid, acetyl bromide and perchloric acid and incubated at 80°C for 40 min. After cooling to room temperature, we added a mixture of NaOH/acetic acid to the extraction supernatant. Then we measured the absorbance at 280 nm and calculated the lignin content of each sample according to the standard curve. For total tannins, we added 0.1 g ground sample to 1 mL distilled water and incubated the sample in a water bath at 80°C for 30 min, then centrifuged at 1,200 g for 10 min and added a mixture of sodium tungstate, phosphomolybdic acid phosphoric acid and Na$_2$CO$_3$ solution to the supernatant. Then we measured the absorbance at 760 nm and calculated the total tannins content. To extract alkaloids, we exposed 0.1 g ground sample in a mixture of ethanol and ammonia solution to low-frequency ultrasound waves for 60 min and centrifuged at 12,000 g for 10 min. We added citric acid-sodium citrate buffer, bromocresol green-potassium phthalate monobasic solution and chloroform. After mixing, we used the chloroform layer to measure the absorbance at 416 nm, and calculated the alkaloids content according to the standard curve. For total flavonoids, we shook a mixture of 0.02 g sample and 2 ml ethanol, which we then incubated at 60°C for 2 h, and then centrifuged at 12,000 g for 10 min. We added NaNO$_3$, Al(NO$_3$)$_3$ and NaOH solution to the extract in sequence, measured the absorbance at 510 nm, and calculated the total flavonoids content.

To characterize soil nutrients and soil pH at each sampling site, we collected a soil sample (~ 50 mL) close to the rhizome of each stem to create a pooled sample for each population. At the third quadrat on each transect, we took a photo of the canopy closure above the stem using a digital camera with the lens oriented vertically. We used ImageJ to convert the images into binary forms, with canopy in black and the sky in white, and to calculate percent canopy closure.

**Environmental data**

We obtained recent climate data (the 1970–2000 averages) for all 150 populations from WorldClim 2, at a spatial resolution of 2.5 arc-minutes. We used the 19 bioclimatic variables (accessible at: https://www.worldclim.org/data/bioclim.html), which cover different aspects of temperature and precipitation intensity and their temporal variability.

To characterize soil conditions at all sampling sites, we dried all soil samples at 40°C, sieved them through a < 2 mm mesh and milled them to < 63 µm in a planetary ball mill. We analyzed the following 11 variables: total soil C, N, Calcium (Ca), Potassium (K), Magnesium (Mg) and Phosphorous (P), as well as plant-available fractions of Ca, K, Mg, P and soil pH. We measured total C and N by elemental analysis via thermal combustion and TCD/IR detection of CO$_2$/N$_2$. For total element contents, we digested the samples with a mix of HNO$_3$, HF and H$_2$O$_2$ (4:4:1) in a microwave and measured the elements by ICP-MS.
We tested element recovery of total digestions with certified reference material (BCR2, Columbia River basalt). We extracted plant available elements by the Mehlich 3 procedure (a mix of NH$_4$NO$_3$, NH$_4$F, HNO$_3$, EDTA and CH$_3$COOH) and measured them by ICP-OES. We measured the soil pH of the sieved material in water (1:2.5).

**Statistical analysis**

We conducted all analyses in R version 4.3.1 $^{57}$. Prior to the analyses, we examined the relationships between pairs of phenotypic traits with Pearson correlation and arbitrarily discarded one if they were highly correlated with a coefficient $> 0.7$. Each response variable was subsequently analyzed independently, with one exception: to estimate the standing biomass per square meter (cm$^3$/m$^2$) we combined stem density, stem height and stem diameter into a composite variable based on the formula:

$$Standing\text{ biomass} = \frac{h \left( \frac{\varnothing}{2} \right)^2 * 3.14}{3} * \text{ Stemdensity}$$

where $h$ is average stem height in a plot, and $\varnothing$ is the average stem diameter. Essentially the first term calculates average stem volume, which is multiplied by stem density to estimate total stem biomass (cm$^3$/m$^2$) per area.

To visualize climatic differences among ranges, we conducted a standardized and centered PCA (principal component analysis) using the `prcomp` function in the package `factoextra` $^{58}$. We generated a PCA biplot of all populations in the climatic space and evaluated climatic variable contributions to the principal components (Supplementary Fig. 1, Supplementary Table 2). We conducted an analogous set of analyses, using the soil nutrient data to visualize differences in soil nutrients within and among ranges (Supplementary Fig. 2, Supplementary Table 3).

As a first step of our data analysis, we tested for range differences in individual variables. For each response variable, we ran a GLMM with range as a fixed factor and population as a random factor, using the `lme4` package $^{59}$. For variables that displayed a significant range effect ($P < 0.05$), we conducted Tukey post-hoc tests to identify which ranges differed from each other. We used density plots generated in `ggplot2` $^{60}$ to visualize the variation within and among geographic ranges. To test for differences in herbivory and pathogens among ranges, we fitted a negative binomial generalized linear model (glm.nb) with log function and tested for differences in group means by simultaneous contrast tests using the `linfct` and `mcp` functions in the `multcomp` package $^{61}$.

Next, we examined the multivariate structure of our data, potential trait syndromes, and their geographic distributions. We constructed Euclidean distance matrices from scaled and centered mean population traits values and used hierarchical cluster analysis with Ward’s linkage to capture similarity and discontinuity among populations (dist and hclust functions in stats package). We used the clusGap function $^{62}$ in the cluster package $^{63}$ to determine the optimal number of clusters, and we visualized
population similarities and clustering through a phenogram with the *dendextend* package. To understand the contributions of individual variables to the clustering, we ran a linear discriminant analysis (*lda* function in *MASS* package) and plotted the LDA loadings, together with the population clusters, in two-dimensional ordination space, using the *ggor* package. We assessed the differences in each trait across clusters with type II ANOVA using the *car* package, followed by pairwise differences tests (*testInteraction* function, *phia* package). To visualize the relative contributions of variables to specific clusters, we created radial plots, using the *radarchart* function in the *fmsb* package.

Finally, we used the environmental data (climate, soil nutrients, canopy closure) to test for drivers of knotweed variation and compare their relative importance in each range. To avoid collinearity among climate and soil variables, and to reduce the total number of tests, we simplified the 19 bioclimatic variables and 11 soil variables through two principal components analyses, one for climate and one for soil within each range. We evaluated only principal components that explained > 10% of the variation in the data (Supplementary Figs. 1 and 2; Supplementary Tables 2, 3). We then used model selection to identify the most parsimonious models for explaining variation in each response variable. Initially, we tested a set of global models that included only the climate PCs, the soil PCs and canopy cover, or any possible combinations of these, – all either without range, with range (i.e. allowing for different means in each range) or with range interactions (i.e. allowing for different environment relationships in each range). Together with a null model and a range-only model this resulted in a total of 23 alternative models (Supplementary Table 4). We used hierarchical model selection with the library *performance* to rank the models based on their Akaike Information Criterion (AIC) and coefficient of determination ($R^2$). As the most parsimonious models often included range interactions (Supplementary Table 4), we further explored the relative importance of the environmental drivers in each range by fitting separate models for each range that included the effects of climate (within range climate PCs, Supplementary Table 5), soil nutrients (within range soil PCs, Supplementary Table 6) and light availability and then conducted variance partitioning for each trait in each range, using the *partvar* function with 1000 bootstrap replicates in the *partR2* package.

**Results**

**Knotweed performance across ranges**

At the population-level, introduced populations in Europe and the USA produced substantially more standing biomass than native populations in China (Europe: +474%; USA: +300%; Fig. 1, Supplementary Table 7). Plants in introduced populations also differed significantly in most leaf traits: on average, plants in European and USA populations had a higher specific leaf area (Europe + 51%, USA + 70%), but decreased levels of leaf chlorophyll (Europe − 35%, USA − 15%), leaf toughness (Europe − 24%, UAS − 9%), leaf C:N ratio (Europe − 52%, USA − 47%) and leaf lignin (Europe − 23%, USA − 65%). In the USA populations – but not European – plants also had significantly lower levels of leaf tannins (-5%) and
flavonoids (-17%) but higher levels of leaf alkaloids (+ 78%) than Chinese populations (Fig. 1, Supplementary Table 7). There were no significant differences among ranges in leaf thickness.

There were also significant differences between the two introduced ranges: plants from the USA populations had on average lower standing biomass (-43%) and leaf lignin (-34%) but tougher (+ 13%) and more chlorophyll-rich (+ 17%) leaves.

**Damage from natural enemies**

Introduced individuals in Europe and North America experienced significantly less damage by herbivores (Europe: -79% less leaf area lost, USA: -76%) compared to individuals in native populations (Fig. 2A, Supplementary Table 7). In addition, only 14% and 17% of the individuals in Europe and the USA respectively showed pathogen lesions compared to the native range where 91% of the individuals exhibited signs of pathogen presence (Fig. 2B, Supplementary Table 7).

**Multivariate trait syndromes**

The hierarchical clustering analysis identified five clusters of observed knotweed trait combinations based on the trait dissimilarity matrix (Fig. 3; Supplementary Fig. 3, Supplementary Table 8). The first two discriminant axes of the LDA explained ~ 92% of the total variation across clusters (Supplementary Fig. 5). Cluster 1 (blue area and dots in Fig. 3) occurred almost exclusively in the native range. These populations had the highest value of C/N, coupled with high levels of leaf toughness and chlorophyll content, as well as increased levels of digestibility-reducing tannins and lignin. This combination of traits defined a conservative growth strategy, with low nutritional quality (high C/N but low SLA levels) and high physical and quantitative chemical defenses. Cluster 2 (purple area and dots in Fig. 3) mainly occurred in the northern area of the native range but also in North America's middle and southern regions. These included individuals with lower amounts of secondary metabolites but relatively high levels of leaf toughness and intermediate C/N. This combination of traits represented an intermediate level of nutritional quality (C/N levels), with moderate levels of physical defense. Cluster 3 (green area and dots in Fig. 3) and Cluster 4 (red area and dots in Fig. 3) occurred only in the introduced ranges of Europe and the northern part of North America. Both clusters contained populations with high standing biomass values, and low levels of leaf chlorophyll and C/N ratio. The two clusters differed in that plants in Cluster 3 had higher values of SLA and flavonoids content, while plants in Cluster 4 displayed higher levels of leaf toughness and tannins. Hence, these two clusters were characterized by a strategy of high nutritional quality and individual biomass, but differed in ways to resist biotic pressure. Cluster 5 (orange area and dots in Fig. 3) included the least number of populations and only occurred in the middle and southern areas of the USA. They tended to have the highest SLA among the clusters, with relatively high levels of leaf chlorophyll and alkaloid content, intermediate leaf toughness and standing biomass values. This combination of traits is consistent with a classic acquisitive strategy (fast growth), with high nutritional quality (low C/N ratio), and toxins as the chemical defense metabolites (high alkaloid levels).

**Environmental drivers of trait variation**
In the global analyses with combined data from all three ranges, there were generally no single best-fit models for explaining trait variation. Instead, we usually found several top candidate models for each of the traits analyzed (Supplementary Table 4). The candidate models often included range interactions indicating different relationships between environment and knotweed performance in different ranges, and we therefore proceeded with separate statistical models for each range to better understand the local responses.

The range-specific models confirmed that the importance of different environmental factors not only strongly varied among traits but often also among ranges. Nevertheless, some general patterns emerged. In all three ranges, specific leaf area and leaf alkaloid content increased with increasing canopy closure, while leaf C:N decreased with increasing canopy closure in both the USA and native China. However, the best models of environmental drivers typically explained only between ~10–20% of the variation in knotweed performance, leaf traits or leaf secondary chemistry (Fig. 4, Supplementary Table 9).

In China, canopy closure was a strong predictor of SLA and alkaloids which increased with canopy closure, and of leaf lignin, tannins, leaf C:N and leaf toughness which all decreased with increasing canopy closure. In the native range, PC1 climate was associated with a decrease in precipitation and winter temperature while PC2 climate was associated with decreasing summer temperature and increasing isothermality (Supplementary Table 5). Combined, the two climate PCs explained a large portion of the variance in standing biomass, leaf chlorophyll, leaf toughness and leaf flavonoids. PC1 soil was associated with decreasing soil pH and plant-available Ca and Mg fractions while PC3 soil was associated with total available phosphorus in the soil (Supplementary Table 6). Combined the soil PCs explained a large amount of the variance in standing biomass, and leaf characteristics: chlorophyll, toughness, lignins, tannins and flavonoids (Fig. 4, Supplementary Table 9).

In Europe, only specific leaf area and alkaloids content were positively associated with canopy closure. Notably, the best models explained only ~5% of the variance in total biomass. PC1 climate was associated with a decrease in precipitation, PC2 climate was associated with increase in temperature, PC3 climate was associated with a decrease in temperature and precipitation seasonality and the temperature annual range (Supplementary Table 5). Combined, the climate PCs in Europe predicted leaf characteristics: chlorophyll, toughness, C:N, alkaloids, but were less predictive of SLA, lignin and flavonoids. PC1 soil was associated with decrease in soil nutrients (total soil N and C, plant available Mg) and PC2 soil with soil pH and total Mg (Supplementary Table 6). Combined soil PCs predicted leaf characteristics: chlorophyll, toughness, C:N and flavonoids (Fig. 4, Supplementary Table 9).

In North America, like in Europe, specific leaf area and alkaloids content increased with increasing canopy closure, whereas leaf C:N ratio showed a negative association with canopy closure. PC1 climate was associated with an increase in temperature seasonality and temperature annual range and a decrease in precipitation while PC2 climate was associated with decreasing summer temperature and the mean temperature of the wettest quarter (Supplementary Table 5). Combined, the climate PCs in North America strongly predicted leaf chlorophyll, tannins, lignins and flavonoids. PC1 soil was associated with soil
nutrients (N and C, plant-available Mg and Ca) while PC2 soil was associated with soil pH and total soil Ca. PC3 soil was associated with increasing total soil K (Supplementary Table 6). The combined soil PCs strongly predicted standing biomass, leaf area, leaf C:N and leaf alkaloids (Fig. 4, Supplementary Table 9).

**Discussion**

While invasive species provide an opportunity to examine the mechanisms that underlie range expansion and adaptive differentiation 72–74, researchers have failed to identify traits or combinations of traits that universally confer invasiveness 14,75. This lack of universality is not surprising since the advantage of a given trait or trait combinations is context-dependent 15. In this study, we sampled populations of Japanese knotweed across a broad environmental gradient in both the native and non-native ranges to more comprehensively evaluate how trait variation is associated with the successful invasion of one of the world’s most invasive plant species complexes. We found consistent differences in knotweed performance between native and introduced ranges that supported our first hypothesis: invasive plants grew larger with high nutritional value but harbored different combinations of chemical defenses compared to native plants. We identified five major ecological strategies, and differences in abiotic and biotic factors that may be driving some of these patterns of trait variation supporting our second and third hypotheses. Combined, our results suggest that enemy release and novel habitat conditions may have driven the emergence of novel ecological strategies in this global plant invader.

**Trait divergence among ranges**

Many plant species that become invasive in their non-native ranges appear to be common but not aggressive in their native ranges 20,76,77. In a meta-analysis of 53 species, Parker et al. 78 found that on average, individuals were larger, more fecund, and more abundant in their introduced ranges. Further, Dawson et al. 79 showed that globally widespread alien species exhibited greater biomass responses to increased resources. Although this may not be a universal pattern across invasive species, previous work in Japanese knotweed indicated that invasive knotweed plants are larger and more robust than their native counterparts 78. One comparative field study of native populations in Japan versus invasive populations in France found that the individuals in the introduced range grew taller, had larger leaves, exhibited much lower leaf damage by herbivores and had a more pronounced effect on the plant communities than native individuals 45. Another study compared *R. japonica* individuals from the native area of Japan and invasive areas of France under common garden conditions 46. That study found that individuals from the invasive populations had higher belowground biomass and increased leaf toughness as well as increased competitive effects against *Rubus caesius*. However, the study detected no range differences in aboveground biomass or plant height 46.

Here, we attempted a more comprehensive comparison of the introduced range in Europe and North America to the native range in China. The trait shifts we observed support an increase in biomass
production and high SLA in the introduced ranges. These trait values are consistent with a high photosynthetic capacity indicating fast growing invasive individuals compared to natives. Leaf area lost to herbivores and pathogen infection were much lower in invasive populations compared with native ones, offering support for the enemy release hypothesis. The low leaf C:N and reduced investment in quantitative defenses (lignin: Europe and USA, and tannins: USA), have been associated with reduced defense against specialist herbivores. These traits are costly to produce and maintain, and are thought to require a trade-off with investing in growth. Together, our findings add to the growing evidence of reduction in top-down constraint imposed by herbivores on invasive plant growth.

We found support for increased plant vigor in both introduced ranges compared to plants in the native range, but the overall patterns differed slightly between the introduced ranges. An increase in production of alkaloids (qualitative defense) in the USA suggests a potential shift in defense to deter generalists. Unfortunately, we were unable to identify the herbivore community feeding on knotweed stands in these populations, so we cannot directly confirm whether a shift from specialist to generalist herbivores has actually occurred. Individuals in USA populations also had on average lower standing biomass and leaf lignin, but tougher and more chlorophyll-rich leaves. These differences could reflect the different biotic and abiotic environmental conditions of the introduced ranges. However, plants from both introduced ranges suffered very little damage from pathogens and herbivores so it is unclear what might have driven the differences in defense traits at the time that we sampled.

**Changes in plant ecological strategy syndromes**

Invasion biologists have identified suites of plant traits that are associated with invasion, but have not often studied how these traits differed between invasive populations and their native source populations. Our findings are informed by both the leaf economics spectrum and defense syndromes framework. The plant strategies identified by the leaf economics spectrum have explained some important differences in ecology across diverse taxa, even in the context of future climate scenarios. However, the concepts have not been much applied within species, even though the broad latitudinal and thus environmental ranges occupied by some species may require shifts in plant strategies within species. Globally successful invasive species like Japanese knotweed provide an opportunity to explore such shifts on two scales: across broad climatic gradients and between the native and introduced ranges.

Although the leaf economics spectrum defines growth strategies which seem relevant in the context of invasion, Reich acknowledged that the framework does not include traits related to dispersal or ‘colonization’. A useful additional framework should therefore be the plant defense syndromes proposed by Agrawal and Fishbein that consist of nutritional, physical and chemical defense traits. Combining these approaches allows for examining how defense and resistance strategies can vary across biotic and abiotic environmental contexts within the range of a species.
We used these frameworks to identify a conservative strategy, and an intermediate strategy in the native range compared to more acquisitive strategies with shifted defenses in the introduced ranges. In the native range, the conservative strategy of cluster 1 was demonstrated by high investment in physical and quantitative chemical defense, and low nutritional quality (highest C/N). This strategy is consistent with the slow growing and ‘low nutritional quality’ syndromes in previous studies. We also found an intermediate strategy in the native range (Cluster 2). Plants with this strategy had a slightly higher nutritional quality than Cluster 1, and contained an intermediate physical defense level with the least values for qualitative defenses. On the contrary, in the introduced ranges, Cluster 5 was characterized as what might be considered the expected acquisitive and invasive strategy with the highest specific leaf area and leaf chlorophyll content, less ‘specialist’ defense investment (tannins and lignin) and shift to ‘generalist’ qualitative defense (alkaloids). This cluster may be more sensitive to herbivores, and might represent the classic expectation in the introduced ranges of a release from specialist enemy pressure, as well as a shift in defense to generalist herbivores. However, this was the smallest cluster and only consisted of 16 populations in the middle and southern areas of the eastern USA. Clusters 3 and 4 in the introduced ranges were also characterized by high nutritional quality and growth, which may reflect the small enemy pressure that we detected. But each was characterized by investment in different chemical defense metabolites, suggesting the potential to resist different biotic pressure and support for the Shifting Defense Hypothesis. Such a shift is consistent with studies that demonstrate decreased resistance against specialist herbivores in introduced populations, with increased resistance against generalist herbivores. However, in the case of clusters 3 and 4 compared to native clusters (1 and 2), the shift was to increased relative investment in flavonoids and tannins (cluster 3) or just tannins (cluster 4) and reduced lignin. Overall, we found a shift from digestibility-reducing compounds in the native “conservative strategy” to different toxins in the invasive, more acquisitive populations.

**Environmental associations**

Prior work has suggested that climatic suitability defined in the native range may not predict invasion success, but so far studies of climatic suitability and invasion success are limited. Pouteau et al. recently completed a comprehensive evaluation of 1,485 endemic European plant species to characterize their predicted range outside of Europe. They discovered that environmental conditions alone can predict up to two-thirds of successful establishments outside of species’ native range. The populations we investigated differed in several environmental factors that were associated with knotweed performance. Of note, the climate experienced by the native populations we sampled was distinct from those in the European and North American ranges, with higher annual, winter and summer temperatures and wetter summer conditions. The Chinese populations also experienced lower seasonal variation, and lower winter and spring precipitation. Nonetheless, our invasive knotweed populations appeared to occupy a range of climatic conditions that is also found in southeast Asia. For example, mean annual temperature ranged from 4.3 to 19.8°C and annual precipitation ranged from 508 to 2146 mm. In addition, the Chinese sites we sampled were characterized by a reduced soil fertility (total P, N, C) and mildly acidic soil pH compared to non-native sites. These results confirm previous work in the native range which reported that
Japanese knotweeds grow in nutrient-poor habitats, colonizing unstable environments such as volcanic soils where they are regarded as pioneer species. While we captured variation across a very large latitudinal gradient in each of the ranges, it could be that the native populations we sampled did not capture the full range of environmental conditions occupied by native populations. In fact, the source of the European and subsequent North American invasion has been traced to Nagasaki, Japan. Including the Japanese range can be more informative for future work.

Although Japanese knotweed traits were significantly associated with several environmental factors, a substantial fraction of the trait variation remained unexplained in our study. This suggested that other, unmeasured factors such as invasion time, disturbance regimes or genetic characteristics may also play a more important role than in other invasive species. The variation in population performance that we could explain appeared to be driven by different climatic and soil nutrients factors in the different regions. For example, standing knotweed biomass is explained largely by climate factors in China, but by soil factors in North America, while the combination of factors explains < 5% of the variance in standing biomass in European populations. The species are reported to be vulnerable to summer droughts (i.e. sites with less than 500 mm precipitation per year). In our study, decreasing summer heat was associated with increased above ground biomass production in both the native and introduced range in North America. We found that light availability (measured as the percent canopy closure) was among the major factors controlling variation in several knotweed traits, but this was more true in China and Europe, not in North America. In the introduced ranges, Japanese knotweeds often occur in open and sunny sites and their performance is reduced in closed canopy habitats. In our populations, reduced light availability was associated with reduction in most of the leaf traits. This was particularly true in China where the sampled populations were generally more shaded than in Europe or the USA (average canopy closure higher than 50%). Specific leaf area and leaf alkaloids production were exceptions, which increased with increasing canopy closure in all three ranges. Such higher SLA increases the light capture efficiency and is common among many species when grown under reduced light conditions.

Environmental factors associated with increasing leaf nutritional value and fiber content (measured as C:N ratio, leaf toughness and leaf lignin content) also varied across the ranges: i.e., with decreasing seasonality (Europe), the annual and winter precipitation as well as winter and summer temperature (China) and summer heat (USA). These traits were also influenced by soil pH (USA) and soil fertility (China – total soil P, Europe and the USA – total N, C and plant available Mg). Climate and soil play important roles in regulating plant metabolites, as their synthesis requires different soil macronutrients including N, P and K, and they are also dependent on an optimum temperature and water availability. Similarly, leaf qualitative (alkaloids and flavonoids) and quantitative defenses (tannins) also showed variation in response to climate and soil factors within ranges particularly to extreme factors such as drought (Europe) and summer heat (USA). Previous studies have shown that the synthesis of plant metabolic compounds can be altered by different abiotic factors which can act as stressors, resulting in a diverse and changeable response. Combined, these results highlight important
differences in abiotic factors across the different ranges which interact with the clear biotic differences and impinge upon trait combinations that we found in the field.

Conclusions

Our work is consistent with previous work that demonstrated that invasive knotweed is more able to take advantage of resources than local native species in Europe \(^{105,106}\). Here, we show that this increase in acquisitive strategy is also true when compared to other knotweed populations in the native range. Reich \(^90\) argued that the leaf economics spectrum is often considered as only alternative extremes of the spectrum: slow-growing, resource-conservative species versus fast-growing species that rapidly take up resources but are less resource-efficient. But in fact, a range of successful strategies exist in every community. Our work extends the implications of different trait strategies to within species growing under different biotic and abiotic conditions, and defines five discrete combinations that are in line with predictions of the rapid evolution of invasive species. This approach allows for discovery of different strategies that may reflect the local changes in enemy pressure and abiotic conditions.

The rapid changes we documented in invasive knotweed populations are remarkable considering the species is known to have spread largely by clonal fragments with very little genetic diversity \(^{28,50,51}\). A recent study of the invasive salt marsh cordgrass provides an important comparison. Invasive *Spartina alterniflora* appears to have evolved rapidly across clines and local conditions in such a way as to generally recapitulate the response of the native populations when the native and invasive plants are grown in common gardens \(^{107}\). However, in that system the invasive plants were the product of massive amounts of introgression across the diversity of source populations that originally seeded the invasion \(^{108}\). In Japanese knotweed, the dramatic differences in phenotype and changes in association of traits with environmental conditions support the contention that invasive populations could result from a “general purpose genotype” which can accommodate a range of biotic and abiotic conditions through phenotypic or developmental plasticity \(^{83,109,110}\). Whatever the genetic make-up of these populations, we were able to identify different strategies employed by this globally invasive plant depending on context. Further studies will decipher how much of these differences are heritable or associated with genetic differences.

Declarations

Data availability

All relevant data generated in this study were submitted to Figshare upon manuscript submission:

https://figshare.com/s/ee5dcf9a439c76227db5

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Author contributions

MP, OB and CLR conceived the project; BL, OB and CLR raised funding and supervised the project; WZ, PC, MP, ZL, SW, JMM, CR, FE, XZ, JB, YZ, EK, JR, ES and CLR performed the field work and lab work; REI and WZ analyzed the data; REI, WZ, OB and CLR wrote the manuscript with assistance from all authors.

Competing interests

The authors declare no competing interests.

Materials & Correspondence

Bo Li, Oliver Bossdorf and Christina L. Richards share responsibility for correspondence and material requests.

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**Figures**
Figure 1

The distributions of traits within populations in the native range of China (red), and the introduced ranges of Europe (green) and North America (blue). The differences among regions are indicated by chi-square and z-scores. Significant range comparisons are in bold.

Figure 2

A) Leaf area lost to herbivores and an image of herbivore attack in the field and B) number of individuals with signs of pathogen presence in native and invasive knotweed populations and an image of pathogen lesions on leaf.
Figure 3

Geographic locations of the 150 knotweed populations surveyed across China (native range), Europe and North America (both introduced range). The symbol colors indicate the predominant multivariate trait syndrome in each population, and the radial plots show the profiles (= traits means) for each of the five trait syndromes.

Figure 4
Percentages of variance of performance, leaf traits or leaf secondary chemistry explained by climate and soil variables, or by canopy closure, in native Chinese versus invasive European and North American knotweed populations. The gray bars represent variance explained by multiple (partially confounded) variables.

**Supplementary Files**

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- SupplementaryInformation.docx