

Hybridization and Plasticity Contribute to Divergence Among Coastal and Wetland Populations of Invasive Hybrid Japanese Knotweed *s.l.* (*Fallopia* spp.)

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Abstract Japanese knotweed *s.l.* (*Fallopia* spp.) is a highly invasive clonal plant, best known from roadside and riparian habitats. Its expansion into beaches on Long Island, NY, USA, represents a major habitat shift. I surveyed populations from beaches and wetlands and conducted a common garden experiment to test for variation in drought tolerance and phenotype among populations and habitats. All populations were composed mostly of first- and later-generation hybrids. I found significant variation among populations in growth, lamina size, specific leaf area (SLA), and biomass allocation, in both the field and the common garden. Lamina size, growth, and root-to-shoot responded plastically to drought treatment. Wetland populations tolerated drought as well as beach populations. Differentiation in SLA between habitats suggests that some selection for beach genotypes may have occurred. It appears that both hybridization and phenotypic plasticity are contributing to the expansion of *Fallopia* spp. into novel habitat.

Keywords Adaptation · Plasticity · Common garden · Invasive species · Long Island, New York · Polygonaceae

Introduction

Invasion by nonnative species represents a serious threat to the health of coastal ecosystems, on par with pollution, overharvesting, and rising sea level (Williams and Grosholz 2008). Coastal habitats have undergone dramatic changes as a result of invasions by nonnative plants such as hybrid cordgrass (*Spartina* spp.), giant reed (*Phragmites aus-*

tralis), and European beach grass (*Ammophila arenaria*; Amsberry et al. 2000; Chambers et al. 1999; Daehler and Strong 1997; Minchinton et al. 2006; Wiedemann and Pickart 1996). In this study, I describe invasive populations of Japanese knotweed *sensu lato* (*Fallopia* spp.) from beach and wetland habitats on Long Island, NY, USA. Japanese knotweed is a vigorously growing, clonal, herbaceous perennial, native to eastern Asia, with known negative impacts on native plant and animal species (Maerz et al. 2005, Lecerf et al. 2007, Siemens and Blossey 2007, Topp et al. 2008). It is considered one of the most invasive plants in North America and Europe (Bailey 2003; Huebner et al. 2004; Pyšek et al. 2001). Invasion of coastal habitats by Japanese knotweed is particularly alarming because of its poor ability to prevent erosion in riparian zones (Tickner et al. 2001), which could translate into significant ecological impacts in coastal areas. Roadsides and riparian zones are considered typical habitat for invasive Japanese knotweed. Although Zika and Jacobson (2003) discussed coastal populations in the northwestern USA and Richards et al. (2008) recently described coastal salt marsh populations on Long Island, there are no previously published reports of Japanese knotweed on beaches in the eastern USA. A spread from mesic and poorly drained environments into very-well-drained beach environments represents a major habitat shift for Japanese knotweed. The ability to expand into novel habitat conditions raises general questions about the mechanisms that enable invasive species to succeed in nonnative ranges.

The establishment and spread of Japanese knotweed are classic examples of the paradox of invasive species: despite limited genetic variability due to population bottlenecks during establishment, invasive species not only overcome the deleterious effects of the bottleneck but are often more successful than locally adapted species (Allendorf and Lundquist 2003). Some researchers have suggested that the

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need to accumulate additive genetic variation for local adaptation is a prerequisite to invasion and is responsible for the commonly observed lag time between the establishment and spread of an invasive species (Ellstrand and Schierenbeck 2000; Lee 2002). Hybridization has been proposed as an important mechanism for generating heritable variation in invasive species (Ellstrand and Schierenbeck 2000; Galatowitsch et al. 1999; Mooney and Cleland 2001), and recent studies have demonstrated that novel combinations of traits or transgressive trait expression in hybrids can allow plants to expand into habitats that are inhospitable to the parent species (Facon et al. 2005; Fitzpatrick and Shaffer 2007; Johnston et al. 2001; Rieseberg et al. 1999, 2007; Schierenbeck and Ellstrand 2009). Alternatively, phenotypic plasticity may provide broad ecological tolerances and explain the ability of exotic species with low genetic variability to successfully invade new habitats (Baker 1965; Parker et al. 2003; Xu et al. 2003). While a number of studies have demonstrated the importance of broad tolerances and plasticity in the success of invasive species (Collyer et al. 2007; Dybdahl and Kane 2005; Funk 2008; Parker et al. 2003; Rapson and Wilson 1992; Richards et al. 2008; Schweitzer and Larson 1999; Xu et al. 2003), others have shown that rapid contemporary evolution may also contribute to the success of invasive plants (Blair and Wolfe 2004; Collyer et al. 2007; Lavergne and Molofsky 2007; Leger and Rice 2003; Maron et al. 2004; Wolfe et al. 2004). In this study, I used a combination of comparative and experimental approaches to evaluate the importance of adaptation and phenotypic plasticity in the invasion success of Japanese knotweed into a novel beach environment. Understanding the relative importance of these factors is essential for invasive species management, both for predicting how a species will respond to biological control and for predicting their potential ecological impact (Sakai et al. 2001; Stockwell et al. 2003; Williams and Grosholz 2008).

Japanese knotweed *sensu lato* includes the taxa *Fallopia japonica* (Houtt.) Ronse Decraene (synonyms *Polygonum cuspidatum* Sieb. and Zucc. and *Reynoutria japonica* Houttn.), *Fallopia sachalinensis* (F. Schmidt) Ronse Decraene, their hybrid *Fallopia* × *bohemica* (Chrtkova) J. Bailey, and any F2 or backcross offspring (Bailey et al. 2007, 2009). Although hybridization between *F. japonica* and *F. sachalinensis* is uncommon in their native range, native populations of the two species do not form two monophyletic groups and some history of interbreeding is likely (Inamura et al. 2000). The invasion history of Japanese knotweed in its introduced range has been thoroughly reviewed by Bailey et al. (2009). Japanese knotweed was introduced through horticulture to both Europe and North America in the mid-nineteenth century and was well established by the early twentieth century (Barney 2006; Bailey et al. 2009). While less is known

about the introduction history and distribution of Japanese knotweed in North America, evidence suggests that it was similar to Europe (Barney et al. 2006, 2009; Barney 2006; Seiger and Merchant 1997). Japanese knotweed species are known to hybridize in both Europe and North America (Bailey et al. 2007; Hollingsworth et al. 1999; Mandak et al. 2005; Tiebre et al. 2007; Zika and Jacobson 2003). The high rates of hybridization in Europe may have been driven by the absence of male fertile individuals of *F. japonica*, but it is still unknown if male fertile *F. japonica* were introduced to North America (Bailey et al. 2009; Forman and Kesseli 2003). Both European and North American populations produce viable seeds that can establish in the wild (Bailey et al. 2009; Bram and McNair 2004; Forman and Kesseli 2003), but sexual reproduction appears more important in North America, perhaps because ecological conditions are more suitable for seedling establishment (Bailey et al. 2009). Recent molecular and morphological work has confirmed that Japanese knotweed populations in the northeastern USA can occur as mixed-taxon swarms, with a range of phenotypes that includes both parental forms and many intermediate forms (Gammon et al. 2007; Grimsby et al. 2007).

On one hand, the invasion history of Japanese knotweed suggests that broad ecological tolerances and phenotypic plasticity, as opposed to high genetic variability, are responsible for its invasion success. Vegetative reproduction is common in both Europe and in North America, and a striking lack of genetic diversity has been reported in European populations (Hollingsworth and Bailey 2000; Hollingsworth et al. 1998). Although there were multiple North American introductions, these introductions were largely from horticultural specimens, suggesting that North American populations may have been founded by a limited number of genotypes (Barney 2006; Barney et al. 2006; Seiger and Merchant 1997). On the other hand, sexual reproduction, including hybridization and introgression, has led to increased genetic diversity in Europe (Bailey et al. 2009) and may have increased the genetic diversity of Japanese knotweed in North America (Bram and McNair 2004; Forman and Kesseli 2003; Gammon et al. 2007). This would allow populations to respond to varying environmental pressures through adaptive evolution. Although earlier studies reported fairly low levels of genetic variation for European populations of Japanese knotweed, these studies only measured neutral variation. Such neutral variation may be a poor indicator of heritable variation for ecologically important traits (McKay and Latta 2002). For example, in roadside and salt marsh populations of Japanese knotweed on Long Island, Richards et al. (2008) found considerable phenotypic differentiation based on traits measured in a common garden, despite limited genetic variation using neutral amplified fragment length polymorphism (AFLP) markers.

To determine if hybridization in North America may have allowed for genetic diversification and adaptive evolution of Japanese knotweed populations on Long Island, I carried out a greenhouse common garden experiment and field surveys to look for variation among populations. If there is phenotypic similarity among populations in both the field and the common environment, then broad tolerances are likely to be a key factor in Japanese knotweed invasion, with a limited contribution from phenotypic plasticity. In contrast, if populations are distinct from each other in the field but similar in the common environment, then plasticity has likely played a role in invasion success. Finally, if populations are phenotypically distinct in the common environment and the field, then genetic differentiation could have played a role in invasion success. However, population differentiation does not necessarily support the presence of adaptive evolution. Such variation can arise from neutral sources such as founder effects and genetic drift, so that individual populations still depend on plasticity and broad tolerances to survive (Keller and Taylor 2008).

To determine if there has been adaptive evolution, I compared populations from beach sites with populations from wetland sites that represent more typical Japanese knotweed habitat. Because the low soil water availability at sandy beach sites could impose a strong selective force for drought tolerance (Kellman and Roulet 1990; Maun 1994; Salisbury 1952), I carried out a greenhouse experiment to test for variation in leaf traits and drought tolerance (measured as growth and biomass allocation) among populations and habitats. In its native range, Japanese knotweed generally inhabits riparian zones or other mesic areas, but *F. japonica* is also a primary colonist of volcanic soils in Japan (Zhou et al. 2003). As a result, there may be preexisting genetic variation in invasive populations for traits that allow plants to tolerate extremely well-drained, nutrient-poor soils, such as the ratio of root-to-shoot biomass (root/shoot) or specific leaf area (SLA; Knight and Ackerly 2003; Mokany et al. 2006; Ordonez et al. 2009). I analyzed variation in leaf size among populations because leaf size is associated with moisture and nutrient availability (Dudley 1996; Wilf et al. 1998) and correlates with many other leaf functional traits (Dunbar-Co et al. 2009; Niinemets et al. 2007). Furthermore, leaf size is known to vary between the two parent species and take on intermediate values in hybrids (Gammon et al. 2007). This taxonomic variation, coupled with the functional significance of leaf size, suggests that leaf size variation could be important for adaptive evolution. Salt tolerance could also be important for success in beach habitats, but, despite significant variation among individuals, there appears to be little variation for salt tolerance among Long Island salt marsh and roadside populations of Japanese knotweed (Richards et al. 2008). Significant phenotypic differences

between beach and wetland populations in a common garden or significantly higher drought tolerance in beach populations would support the hypothesis of adaptive evolution to the edaphic conditions found in beach habitats.

Materials and Methods

Field Sites and Study Organisms

This study encompasses eight different field sites in Suffolk County, Long Island, NY, USA (Table 1). I chose sites that were geographically dispersed throughout the county and represented the range of beach and wetland habitats found in this area. On Long Island, beach habitat consists of sites along the north shore, adjacent to Long Island Sound, represented by HLB, HPL, and PJB, and sites along the south shore adjacent to the Atlantic Ocean, represented by ANW. Along the north shore, Japanese knotweed grows in the backshore and in sand at the foot of a bluff, with some plants extending up the bluff. Japanese knotweed is less common on south shore beaches, where it grows in intertidal areas. Japanese knotweed grows in a variety of wetland habitats on Long Island, from which I sampled riparian zones (GCP and PJP), a low-lying area surrounded by forest (SNW) and a salt marsh (WNW). Although these wetland areas are ecologically diverse, they all have consistently wet soil, which should reduce any selection for drought tolerance. Some populations occurred as contiguous stands, while others were comprised of multiple stands. Riparian and beach populations were roughly linear, with the remaining populations covering a roughly rectangular area. The approximate area occupied by each population is given in Table 1. Unfortunately, there are no historical records to indicate the age of these populations. I originally selected three beach (ANW, HPL, and PJB) and three wetland (GCP, SNW, and WNW) populations for the field and greenhouse studies. Two of the original sites, one wetland (SNW) and one beach (ANW), were mowed during the summer and were inappropriate for the collection of phenotypic data. They were replaced for field study by two additional sites (PJP and HLB).

At each site, I collected soil from three randomly selected locations directly under Japanese knotweed plants, from 10 cm below the surface. I measured gravimetric water content, the ratio of the mass of water in the soil to the mass of dry soil. I measured water content at the original six sites 1 day after rainfall and at all eight sites 1 week after rainfall. I compared gravimetric water content among sites and between habitats using the GLM procedure in SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA), testing the effect of site nested within habitat [site (habitat)] over the error sum of squares mean square error (MSE) and the effect of habitat over the site (habitat) MSE.

Table 1 Names, locations, and habitats of field sites used in the study of Japanese knotweed in Suffolk County on Long Island, NY, USA

Name	Code	Habitat	Location	Latitude	Longitude	Population dispersion	Approximate area occupied by populations (m)
Wertheim National Wildlife Refuge	WNW	Marsh	Shirley	N 40° 46.228'	W 72° 53.858'	Contiguous	40×60
Givens County Park	GCP	Riparian	Smithtown	N 40° 51.368'	W 73° 12.610'	Multiple stands	10×200
Seatuck National Wildlife Refuge	SNW	Wet forest clearing	Islip	N 40° 42.916'	W 73° 12.626'	Contiguous	50×70
Port Jefferson Park	PJP	Riparian	Port Jefferson	N 40° 56.614'	W 73° 04.384'	Contiguous	20×200
Amagansett National Wildlife Refuge	ANW	Back dunes	Amagansett	N 40° 58.152'	W 72° 07.682'	Contiguous	30×50
Hagerman Landing Beach	HLB	Beach/bluff	Sound Beach	N 40° 57.970'	W 72° 57.279'	Multiple stands	50×300
Horton Point Lighthouse	HPL	Beach/bluff	Horton Point	N 41° 05.169'	W 72° 26.675'	Multiple stands	20×300
Port Jefferson East Beach	PJB	Beach	Port Jefferson	N 40° 57.826'	W 73° 02.826'	Multiple stands	30×500

Area occupied by populations is the area over which a population was dispersed, not the total area occupied by Japanese knotweed plants

I used morphological characteristics to identify the taxa at each field site (Bailey et al. 1995; Gammon et al. 2007; Zika and Jacobson 2003). This method has been used to identify Japanese knotweed taxa in Europe and North American and produces classifications that are consistent with molecular markers for each taxon (Bailey et al. 1995; Gammon et al. 2007). I only scored trait values in the range for *F. japonica* and *F. × bohemica* because I never observed *F. sachalinensis* during numerous surveys of potential field sites (personal observation), despite reports of its occurrence on Long Island (Weldy et al. 2005). I scored leaves from eight to ten plants at each site for length from base to tip (1: <18 cm, 2: 18–20, 3: >20 cm), trichome size (1: glabrous, 2: scabrous or tuberculate, 3: muricate or palpillate), shape of base (1: acuminate, 2: truncate, 3: cordate), and shape of tip (1: cuspidate, 2: intermediate, 3: acuminate). In each case, the first character is indicative of *F. japonica* and the third character is indicative of *F. × bohemica*. A score of 2 for trichome size or shape of base is consistent with *F. japonica*, while a score of 2 for length or shape of tip is ambiguous. If all four characteristics for a leaf were consistent with one taxon, the plant was assigned to that taxon; otherwise, it was considered ambiguous. I used logistic regression to determine if there was a significant difference in the taxonomic composition among sites (Fit Model module in JMP v.5.1, SAS Institute Inc., Cary, NC, USA). Since there were no differences in taxonomic composition among populations (see “Results”), I did not consider taxon as an effect in other analyses.

Field Measurements

I measured stem density, aboveground biomass, lamina area, and SLA (lamina area divided by dry mass) at three wetland sites and three beach sites. Each site was harvested on a separate day during the last 2 weeks of August 2003, with

the harvest dates chosen randomly. At each site, I randomly chose five 0.5×0.5-m plots and counted the number of stems per plot. This number was multiplied by four to get the stem density in stems per square meter. From the same five plots, I harvested all aboveground biomass. I sorted the biomass into support tissue (stems and petioles), laminas, and reproductive tissue. Reproductive tissue included the entire inflorescence. I dried all tissue to constant weight at 60°C and weighed it. To calculate lamina area and SLA, I collected approximately 50 sun-exposed leaves from each study site, using only leaves of the same developmental stage (seventh or eighth leaf from the tip of the stem). Each leaf was collected from a separate stem separated by at least 1 m. Since nearby stems may be ramets of the same genotype, this sampling method may have led to some pseudoreplication, so *P* values are interpreted conservatively. I scanned fresh leaves using a Canoscan 8000F flatbed scanner (Cannon USA Inc., Lake Success, NY, USA) and calculated lamina area using Scion Image Software (Scion Corporation, Frederick, MD, USA). After scanning, I dried leaves at 60°C, weighed them, and calculated SLA.

Greenhouse Experiment

I carried out a greenhouse experiment to examine the same traits I measured in the field under common environmental conditions, in order to determine if variation among sites had a genetic basis and to assess how plants responded to drought. I collected rhizomes from three beach sites and three wetland sites and grew the plants in a greenhouse at Stony Brook University in Stony Brook, NY, USA. At each site, I collected ten separate rhizome pieces from locations at least 10 m apart. I cut each rhizome into two pieces weighing 5 (±0.6) g and randomly assigned each half to control or dry treatment. I sprouted rhizomes in flats and then planted sprouted rhizome pieces into 4-l pots, in a mixture of six parts Turface (Profile Products, Buffalo Grove, IL, USA), two parts play sand, and

one part vermiculite. Control plants were watered as needed to maintain the medium at field capacity. Dry-treatment plants were kept moist until the cuttings sprouted (6–12 day) and then watered only when the medium had dried out to at least 5 cm below the surface. Plants were arranged on the greenhouse bench in a random order. The rhizomes were planted on August 5, 2003 and maintained until harvest 8 weeks later.

On October 3, 2003, I collected two fully expanded leaves from each plant for analysis. Leaves were scanned, dried, and weighed, and the average lamina area and SLA of the two leaves per plant were calculated as described above. On October 5 and 6, 2003, I harvested the aboveground biomass of all greenhouse plants. Stems were cut at the soil level, and tissue was divided into leaves and stems. For the few plants that were flowering, reproductive tissue made up a very small part (<1%) of the plant biomass and was included with stem tissue. Leaves and stems were dried at 60°C and weighed. Pots were kept in a cool, dark location after harvest, and root tissue was harvested between October 7, 2003 and October 15, 2003. After rinsing with water, roots were dried and weighed as described for stems.

Data Analysis

Field Data Since leaves were collected across whole sites rather than by plot, I analyzed leaf data and plot data separately. All transformations and exploratory statistical analyses were performed with JMP v. 5.1.2 (SAS Institute Inc., Cary, NC, USA). Lamina area and SLA were transformed with the Box–Cox algorithm to improve normality (Sokal and Rohlf 1995). The correlations between lamina area and SLA did not vary greatly or change signs between sites or habitats, suggesting that the assumption of multivariate normality was valid (Scheiner 2001). The data for stem density, stem mass, lamina mass, and reproductive biomass were transformed using the Box–Cox algorithm. Because field biomass data were collected on six separate days over the course of 2 weeks, with each site being harvested on a separate day, site may have been confounded with date of harvest. I used linear and quadratic regression to determine if there was a relationship between date of harvest and transformed biomass measurements. Only transformed reproductive biomass was significantly correlated with date of harvest ($r=0.50850$, $P=0.004$), since plants were in the reproductive phase and there was little vegetative growth during the period of harvest. To remove the effects of date of harvest on reproductive biomass, I used quadratic regression to fit the transformed reproductive biomass to the date of harvest and used the residuals from the regression in subsequent analyses. Untransformed stem and lamina biomass were strongly correlated ($r>0.95$ for all sites), so I combined stem and lamina biomass into one measure (stem + leaf mass) by summing them and transforming the sum. I calculated the

proportion of biomass in stems as the stem biomass divided by stem + leaf mass. This parameter evaluates investment in support versus assimilative tissue. I did not include reproductive biomass in the denominator because of the confounding effect of date. Both stem+leaf mass and proportion stem were transformed using the Box–Cox algorithm.

Greenhouse Data For the greenhouse experiment, leaf data and biomass data were taken from the same plants and considered as one data set. There were strong correlations between the transformed stem, lamina, and root biomass ($r>0.85$ for all contrasts for all sites), so I created a new variable total mass, by summing the stem, lamina, and root biomass. I calculated the proportion stem as the stem mass divided by the stem plus lamina mass. I calculated root/shoot as root biomass divided by stem and lamina biomass. Box–Cox transformations were applied as necessary to improve the normality of response variables.

Statistical Tests For each data set (field leaf data, field plot data, and greenhouse data), I conducted a multivariate analysis of variance (MANOVA) using the GLM procedure in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). I conducted a univariate analysis of variance (ANOVA) for each response variable, for comparison with the MANOVA results. For field leaf data, I used lamina area and transformed SLA as response variables, with habitat and site (habitat) as factors. For the field plot data, I used stem density, stem + leaf mass, reproductive residuals, and proportion stem, with the transformations described above, as response variables, and habitat and site (habitat) as effects. For the greenhouse experiment, I used lamina area, SLA, total biomass, and root/shoot, with the transformations described above, as response variables and habitat, site (habitat), treatment, and two-way interactions between treatment and the other effects as effects. For both MANOVAs, the effect of site (habitat) was tested over the error sum of squares and cross-products (SSCP) matrix and the effect of habitat was tested over the site (habitat) SSCP matrix. Treatment and habitat × treatment were tested over the treatment × site (habitat) SSCP matrix, and treatment × site (habitat) was tested over the error SSCP matrix (Quinn and Keough 2002; Rencher 2002).

Results

Taxonomic Identification and Differences Among Field Sites

There were no differences in taxonomic composition among the populations or between habitats [site (habitat):

Wald $\chi^2=4.3856$, 8 *df*, $P=0.82$; habitat: Wald $\chi^2=0.006$, 2 *df*, $P=0.997$]. Only two plants had all four characters consistent with *F. japonica*; 18% of the plants were scored as *F. × bohemica*, and the majority of the plants (79%) had a mix of characters from *F. japonica* and *F. × bohemica*, suggesting later-generation hybridization or introgression. Each population contained individuals with three to six different combinations of leaf characters. Since there is evidence for a molecular basis for these characters (Gammon et al. 2007), there appears to be multiple genotypes in each population. There do not appear to be any differences between beach and wetland populations in the presence of first- versus later-generation hybrids.

There were significant differences in gravimetric water content among sites, and gravimetric water content was significantly higher at wetland sites ($F=7.5355$, 4 *df*, $P=0.003$ for site (habitat) and $F=27.418$, 1 *df*, $P<0.001$ for habitat, for measurements taken the day after rainfall, $F=3.298$, 6 *df*, $P=0.026$ for site(habitat) and $F=11.982$, 1 *df*, $P=0.003$ for habitat, for measurements taken 1 week after rainfall). There are different degrees of freedom for the two tests because the samples taken the day after rainfall included only the original sites, while the samples taken 1 week after rainfall included the two additional sites (PJP and PJB, Table 1).

Field Data

Statistical output for all tests is listed in “Appendix.” The MANOVA on field leaf data revealed a highly significant effect of site (habitat) ($P<0.001$) but no effect of habitat ($P=0.587$). Since the P values for this and other tests are either very high or very low, it is unlikely that any potential pseudoreplication due to unknowingly resampling the same genotype influenced the results. Lamina area had a greater effect on the differences among sites than SLA, based on the standardized canonical coefficients (“Appendix” Tables 4 and 5). However, the univariate ANOVAs suggest that both lamina area and SLA were significantly different among sites (Fig. 1, Table 2, and “Appendix” Table 6). There was also a highly significant effect of site (habitat) ($P=0.004$) but no effect of habitat ($P=0.300$) in the field plot MANOVA (Fig. 2, “Appendix” Table 7). Stem + leaf mass and the residuals of reproductive mass had the greatest influence on the differences among sites, based on the standardized canonical coefficients (“Appendix” Table 8). The opposite signs of the canonical coefficients imply that they were negatively correlated across sites (Scheiner 2001) and suggest a tradeoff between vegetative and reproductive biomass. For this analysis, the univariate ANOVAs contrasted with the MANOVA results, with none of the response variables showing a significant effect of site (habitat) (Table 2 and

“Appendix” Table 9). The ANOVA for stem + leaf mass indicated that aboveground biomass was higher in beach sites (Table 2, Fig. 2).

Greenhouse Data

Site (habitat) had a significant effect on the outcome of the greenhouse experiment ($P<0.001$, “Appendix” Table 10). Consistent with the field data, habitat did not affect the response variables when they were analyzed together ($P=0.292$). Although treatment did not have a significant multivariate effect ($P=0.176$), there was a significant interaction between treatment and site (habitat) ($P=0.001$), suggesting that there was variation among sites in response to the drought treatment (Fig. 3). Based on the standardized canonical coefficients, lamina area had the greatest impact on the difference among sites, and root/shoot had the greatest impact on the treatment \times site (habitat) interaction (“Appendix” Table 11). All univariate ANOVAs were significant overall (“Appendix” Table 12). Site (habitat) had a significant effect on lamina area, SLA, and total biomass (Table 3). Although there was no significant multivariate effect of treatment or habitat in the MANOVA, in univariate tests, treatment had a significant effect on lamina area, total biomass, and root/shoot, and habitat had a significant effect on SLA. There was a significant univariate treatment \times habitat interaction for lamina area. The treatment \times site (habitat) interaction was not significant for any individual traits, despite its multivariate significance. As with the field data, significant P values were well below 0.05, so it is unlikely that pseudoreplication affected the results.

Discussion

In a comparison of Japanese knotweed populations from beach and wetland habitats on Long Island, NY, USA, I found that all populations were composed almost entirely of first- and later-generation hybrids. Significant differences in the common garden provide evidence for genetic variation among populations in leaf morphology, growth, and biomass allocation patterns, and the significant treatment \times site(habitat) interaction demonstrated that populations responded differently to imposed drought (“Appendix” Tables 10, 11, and 12). Despite the variation among populations in both the field and the greenhouse, the evidence for adaptation to beach or wetland habitats was weak. Habitat had no overall effect in any of the MANOVAs, indicating that both beach and wetland populations were equally sensitive to drought. There were significant univariate effects of habitat on several individual traits, suggesting that some differentiation according to habitat may have

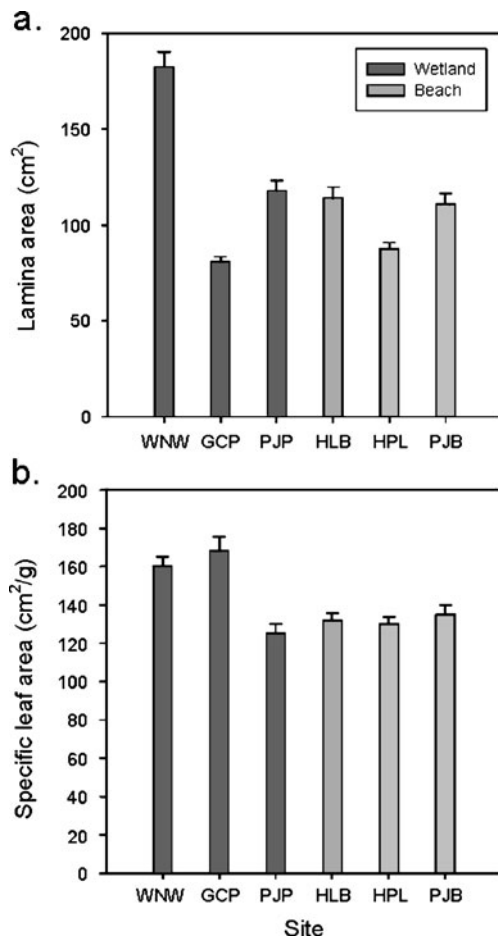


Fig. 1 **a** Mean lamina area and **b** specific leaf area, plus SE of Japanese knotweed measured at six field sites in two habitats on Long Island, NY, USA (N varies by site; WNW 49 leaves, GCP 56 leaves, PJP 59 leaves, HLB 57 leaves, HPL 55 leaves, PJB 60 leaves)

occurred. There was also considerable plasticity for ecologically important traits like lamina area, SLA, or root/shoot (Fig. 3). This may help Japanese knotweed establish and survive in beach habitats. The combination of plasticity and phenotypic variation is likely to increase the invasion potential of introduced populations via adaptive evolution.

Hybridization as a Source of Variation

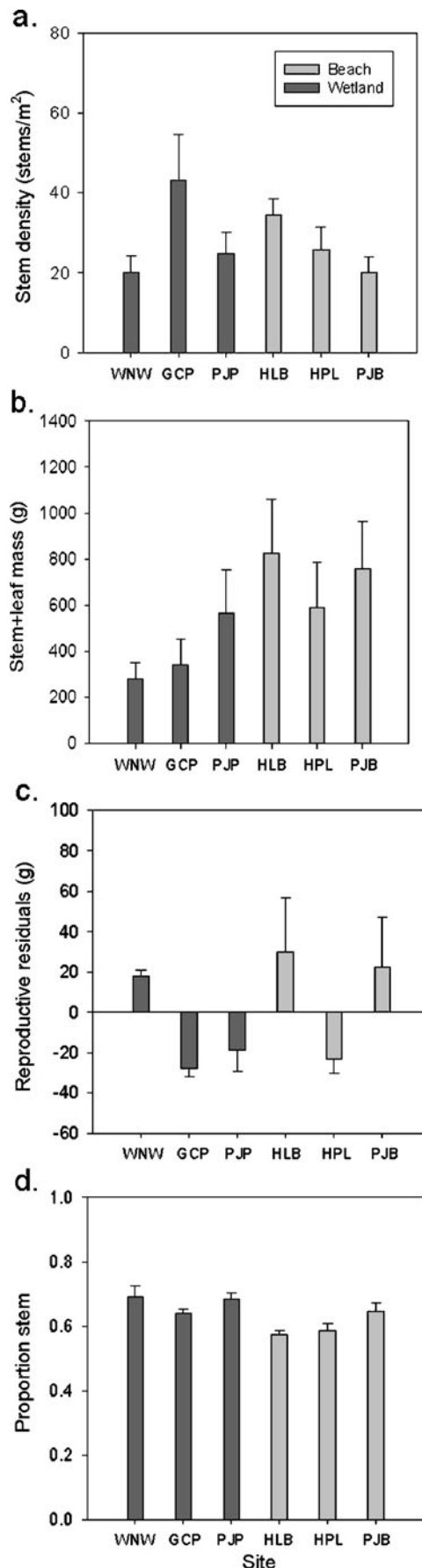
Interspecific hybridization has long been recognized as a source of genetic diversity (Anderson 1949; Ellstrand and Schierenbeck 2000; Lewontin and Birch 1966), often resulting in a range of trait values for the offspring that exceeds that of the parent taxa (Burke and Arnold 2001; Parnell et al. 2008; Rieseberg et al. 1999). Extreme trait values or novel combinations of traits may allow hybrids to invade new habitat (Abbott 1992; Baumel et al. 2001; Facon et al. 2005; Lexer et al. 2003). For invasive species with low genetic diversity, hybridization can provide

Table 2 Summary of statistical output for the univariate tests of the effects of habitat and site nested within habitat on Japanese knotweed plants at six field sites on Long Island, NY, USA

	ANOVA P values	
	Habitat	Site(habitat)
Lamina area	0.538	<0.001
SLA	0.309	<0.001
Stem density	0.937	0.083
Stem + lamina mass	0.049	0.614
Reprod. mass resid.	0.321	0.137
Proportion stem	0.064	0.099

Italicized text is used to indicate significant P values. Full statistical output is available in “Appendix” Tables 4, 5, 6, 7, 8, and 9

sufficient variation for adaptive evolution to proceed (Ellstrand and Schierenbeck 2000; O’Hanlon et al. 1999; Suehs et al. 2004). Previous studies of European populations of Japanese knotweed have reported very low genetic diversity (Hollingsworth and Bailey 2000). However, recent hybridization among the three species and *F. × bohemica* has led to increased genetic variation in Europe (Mandak et al. 2005; Pyšek et al. 2003; Tiebre et al. 2007). There is evidence of sexual reproduction and hybridization (including backcrosses) in American populations of Japanese knotweed, suggesting that genetic diversity could be higher in the USA (Bram and McNair 2004; Forman and Kesseli 2003; Gammon et al. 2007; Grimsby et al. 2007). In my survey of beach and wetland populations of Japanese knotweed, I found that all populations contained mostly individuals that were morphologically classified as *F. × bohemica* and either backcrosses or F2 hybrids (those scored as ambiguous), and even those scored as *F. × bohemica* could represent later-generation hybrids or backcrosses (Gammon et al. 2007). There were only two morphologically pure *F. japonica* genotypes. There was no evidence for transgressive trait expression in hybrid Japanese knotweed in the populations I surveyed or elsewhere in northeastern North America (Gammon et al. 2007). Instead, it appears that sexual reproduction and hybridization led to segregation of the variation present in the parent taxa, producing phenotypically variable offspring with intermediate forms. For example, *F. japonica*, *F. × bohemica*, and *F. sachalinensis* have small, medium, and large leaves, respectively (Gammon et al. 2007; Zika and Jacobson 2003), so hybrid offspring of these taxa should have highly variable leaf size. Consistent with this expectation, I found that lamina area was significantly different among populations both in the field and in the common garden (Tables 2 and 3). Since all populations were taxonomically similar, the differences among populations in this study were not due to differences in taxonomic composition but instead suggested idiosyncratic selection or



◀ **Fig. 2** a Mean stem density, b stem plus leaf mass, c residuals of reproductive mass regressed on date of harvest and d proportion stem, plus SE, of Japanese knotweed measured at six field sites in two habitats on Long Island, NY, USA ($N=5$ plots per site)

founding events leading to a unique mix of genotypes in each population. Although I was careful to start with rhizome pieces of similar size for all populations, I cannot rule out maternal or other epigenetic effects that may have been carried over from the field sites (Rapp and Wendel 2005).

My experimental design did not allow for a statistical test of phenotypic or genetic variation among genotypes within each population. My observations suggest that it was present but limited. In the common environment, there were clear differences in leaf color, leaf shape, growth habit, flowering time, and other traits that made it easy to distinguish plants from different populations, and those traits did not vary much within populations. The significant effect of population on plant phenotype also requires lower within than among population variation. Nonetheless, there was some variation within populations, especially for root/shoot (see error bars in Fig. 3). Each population contained individuals with at least three different combinations of taxonomic traits, suggesting a minimum of three genotypes per populations. In their recent study of Long Island Japanese knotweed populations, Richards et al. (2008) found little or no AFLP variation within populations but still observed significant phenotypic variation among individuals within populations in a common garden, for traits such as biomass and succulence. Although I cannot rule out the possibility of having sampled multiple ramets from the same clone within each population, my sampling design was similar to Richards et al. (2008) and thus likely to have similar levels of intrapopulation variation.

Unlike this study, Richards et al. (2008) did not find significant differences among sites for plants grown in a common garden. However, they did report that adjacent Japanese knotweed populations had the same AFLP genotype regardless of habitat, while geographically separated populations had different AFLP genotypes. This is consistent with the pattern I found since all of my populations were geographically distant. The significant variation among but apparently limited variation within populations suggests that establishment by seed was probably important for the founding of each population, with both clonal spread and sexual reproduction contributing to population growth. Grimsby et al. (2007) recently found the same pattern in a survey of Massachusetts populations of Japanese knotweed, using simple sequence repeat markers.

In the common garden, I found significant variation among sites for lamina area, SLA, and total biomass. I found significant variation in reproductive biomass in the field, but I could not measure this trait in the greenhouse to determine if that variation was genetic.

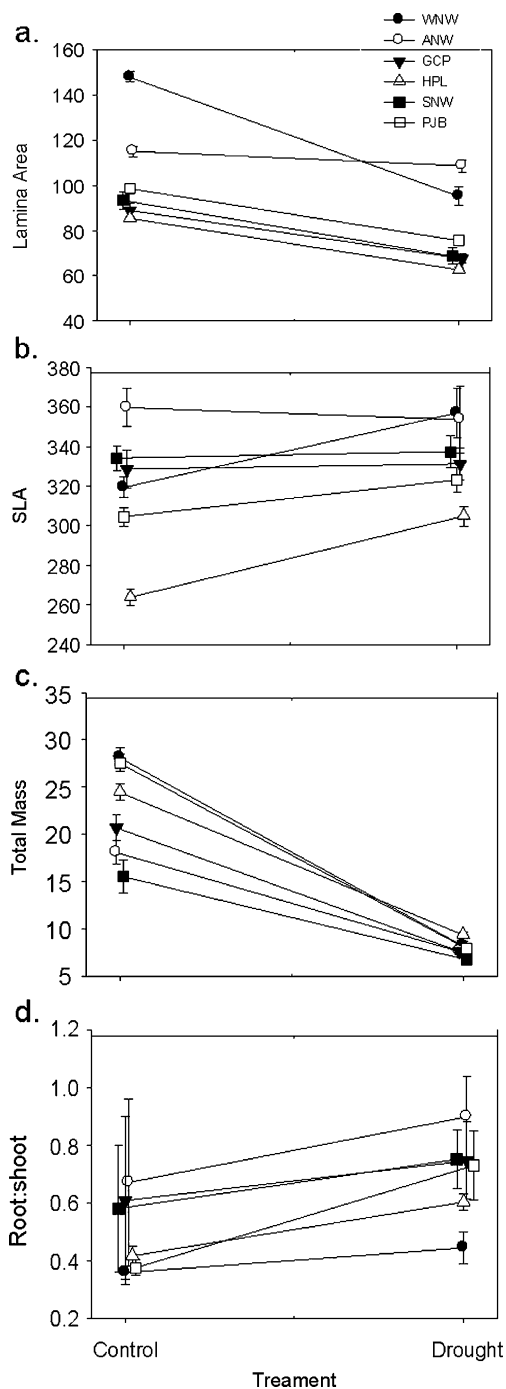


Fig. 3 Reaction norms for **a** mean lamina area, **b** specific leaf area, **c** total mass, and **d** root/shoot, plus/minus SE, for Japanese knotweed grown from cuttings from six sites and two habitats, grown in the greenhouse under control or drought conditions (N ranged from 6 to 10, depending on the population and treatment). Filled symbols represent wetland populations and open symbols represent beach populations

These traits illustrate the many ways that hybrid offspring can differ to provide the phenotypic and genetic variation on which selection could act. Traits such as lamina size and SLA have known functional

Table 3 Summary of statistical output for the univariate test of the effects of drought treatment, habitat, and site nested within habitat on Japanese knotweed plants in the greenhouse

	ANOVA P values			
	Lamina area	SLA	Total mass	Root/shoot
Model	<i><0.001</i>	<i>0.008</i>	<i><0.001</i>	<i><0.001</i>
Habitat	0.475	<i>0.019</i>	0.151	0.150
Site (habitat)	<i><0.001</i>	<i>0.021</i>	<i>0.030</i>	0.172
Treatment	<i><0.001</i>	0.186	<i><0.001</i>	<i><0.001</i>
Treatment \times site (habitat)	0.065	0.173	0.221	0.659
Treatment \times habitat	1	0.861	0.901	0.574

Italicized text is used to indicate significant P values. Full statistical output is available in “Appendix” Tables 10, 11, 12

significance and suggest that individual populations are functionally different from each other. Both within and among species, lamina size correlates with water availability, and SLA is an indicator of physiological functions such as photosynthesis and respiration (Dudley 1996; Reich et al. 1998; Wilf et al. 1998). Differences among populations in total biomass and reproductive biomass suggest a high potential for variation in reproductive output and fitness. If that variation is heritable, this could eventually lead to a shift in dominance of particularly fit genotypes. The functional variation present in Long Island Japanese knotweed suggests not only high potential for evolutionary change but also the possibility of variable impacts among populations. Since Japanese knotweed can dominate plant communities in the areas it invades, differences in functions like gas exchange or growth rate could translate into significant difference in ecosystem-level properties such as net primary production or transpiration.

Natural Selection and Response to Drought Treatment

The high variation among sites and the lack of any significant effect of habitat suggest that site-specific factors—either variable selection at each site or chance events during the establishment of each population—are driving the divergence of populations. Given the small population sizes and putatively young age of the populations, selection would have to have been very strong and occurred during initial establishment to result in such divergence. Selection of this sort has been demonstrated for other clonal plants. For example, North American coastal populations of *Spartina patens* displayed adaptive genetic divergence among adjacent stands on dunes, swales, and marsh sites (Silander 1979). Likewise, invasive populations of *Spartina alterniflora* on the Chinese coast contain molecular markers specific to each of three habitat

types (Deng et al. 2007). Deng et al. suggest that selection acted on *S. alterniflora* seedlings during establishment and led to habitat specialization. Those studies found variation among habitats, however, and I found variation within each habitat type. Divergent natural selection for individual populations within a habitat could have occurred if there were divergent selection pressures among sites within each habitat. This is plausible for the wetland sites that included a salt marsh, a riparian zone, and a wet forest clearing but seems less likely for the beach sites. Beach sites were ecologically very similar yet showed as much among population variation as wetland sites. If there were habitat-based selection occurring at the population level, I would expect beach populations to show some convergence, at least in the phenotypes of plants growing in the field. Rather than site-specific selection, the pattern of high variation among and low variation within sites suggests that populations were founded by a few seeds and/or clonal propagules, followed by clonal spread within each population.

Despite the lack of an overall effect of habitat in either the field or the greenhouse, there were responses in some individual traits. In the greenhouse, SLA was significantly different among habitats (Table 3), suggesting that this trait may have diverged genetically between habitats. Two of the three beach populations had lower SLA than wetland populations when grown in the common environment (Fig. 3), and, although the effect was not significant, SLA of beach populations measured in the field was equal to or lower than that of all wetland populations (Fig. 2). Lower SLA, which indicates thicker or denser leaves, can confer drought tolerance because those leaves have a higher proportion of their mass in cell wall tissue and are able to withstand lower water potentials (Cano et al. 2008; Martinez et al. 2007; Mitchell et al. 2008). Lower SLA in beach habitats could also be a response to generally higher light levels (Evans and Poorter 2001) since the beach habitats were very open, while the wetland habitats (excluding the salt marsh) experienced partial shade. Nonetheless, the population with the highest SLA in the common environment was a beach population (ANW), so clearly there has been no uniform selection for lower SLA in beach environments.

The response to drought treatment in the greenhouse experiment suggests that both beach and wetland populations are equally sensitive to drought. Different populations responded differently to drought, as indicated by the significant treatment \times site (habitat) interaction, but there was no overall habitat effect or treatment \times habitat interaction (“Appendix” Table 10). All populations had reduced lamina area, lower total biomass, and increased root/shoot under drought (Fig. 3). There was a significant univariate habitat \times treatment interaction for lamina area

(Table 3), but this appears to be driven primarily by the responses of two populations. WNW, a wetland population, had an exceptionally large decrease in lamina area, while ANW, a beach population, had a smaller than average reduction (Fig. 3). Although this result is consistent with an increased sensitivity to drought in one wetland population and a decreased sensitivity in one beach population, most of the populations had almost identical changes in lamina area, regardless of their habitat. As with other traits in this study, it appears that any selective evolution for drought tolerance has occurred at the level of the population and not the habitat.

Although my results demonstrated that Japanese knotweed plants from beach and wetland sites are equally sensitive to drought when grown in pots, field-grown plants may have mechanisms that allow them to avoid drought stress rather than tolerate it. The lack of a difference in lamina area between beach and wetland populations in the field supports the idea that beach-grown plants do not need to tolerate drought because they are avoiding it. Field-grown plants have extensive rhizomes and large, succulent stems that may be used to buffer the plant from water loss during periods of rapid transpiration (Meinzer et al. 2001), whereas greenhouse-grown plants had no substantial rhizome development and limited stem growth during the course of the experiment. Leaf succulence could also be important since Richards et al. (2008) found significant variation for leaf succulence among individuals in a common garden. Plants at beach sites may also have deeper roots and greater belowground biomass than those at wetland sites, further helping field-grown beach plants to avoid drought. The greenhouse experiment suggests that there is no genetic variation among sites or habitats for root/shoot, but there was significant plasticity in this trait (see below), which could be important in the field.

The Role of Phenotypic Plasticity and Broad Ecological Tolerances

In contrast to genetic diversity and adaptive evolution, some invasions may succeed due to a preponderance of general-purpose genotypes (Baker 1965; Parker et al. 2003). Earlier selection may give rise to species or populations that have limited genetic variation but are able to tolerate a broad range of ecological conditions through phenotypic plasticity. Given that many invasive species enter their new range with low genetic diversity, plasticity can facilitate invasion by allowing organisms to tolerate harsh or novel conditions (Freeman and Byers 2006; Funk 2008; Muth and Pigliucci 2007; Parker et al. 2003; Richards et al. 2006). In the case of Japanese knotweed, phenotypic plasticity may facilitate the spread

into coastal habitats since there is no evidence to adaptive evolution for this novel habitat. All populations showed plasticity in response to drought, although the amount was variable (Fig. 3). Some plastic responses to drought, such as reduced biomass and lamina area, arise because of the high sensitivity of stomatal conductance, cell elongation, and protein synthesis to low water potential (Lambers et al. 1998). This may reduce reproductive output but help plants survive under drought conditions. Greater sensitivity of aboveground than belowground tissue leads to an increased root/shoot and increases the ability to take up water relative to the amount of transpiring tissue (Lambers et al. 1998).

The phenotypic breadth of characters measured in the field may arise from genetic variation, or it could be an expression of phenotypic plasticity. There was a marginal effect of habitat on proportion stem in the field ($P=0.064$, Table 2), with no effect in the greenhouse ($P=0.518$ in a univariate ANOVA, data not shown). This suggests that beach plants may be able to plastically allocate less biomass to support tissue, possibly because of less competition for light. This characteristic would allow them to invest more of their resources in light capture, reproductive, or root tissue, increasing their overall resource use efficiency. There was a significant effect of habitat on stem + lamina mass (equal to aboveground biomass minus reproductive tissue) in the field, but no differences in total biomass in the greenhouse, either at the population or the habitat level (Tables 2 and 3). This suggests that the difference in biomass in the field is a plastic response. Since stem + lamina mass was higher in beach habitats than wetland habitats, beach habitat actually may be better for Japanese knotweed growth than wetland habitat. Plasticity in response to habitat variation may compliment the genetic variation arising from hybridization, with both characteristics contributing to invasion success.

Conclusions

The invasive species literature contains many examples of previously reproductively isolated species or populations that interbreed in their invasive range, leading to highly successful invasive populations, either through the generation of genetic novelty or fixed heterosis (Abbott et al. 2003; Bleeker 2003; Ellstrand and Schierenbeck 2000; Facon et al. 2005; Gaskin and Schaal 2002; Lexer et al. 2003; Pooler et al. 2002). Earlier studies of invasive Japanese knotweed reported evidence of low genetic diversity and clonal reproduction (Hollingsworth and Bailey 2000; Hollingsworth et al. 1998). While some particularly widespread genotypes continue to be found in

both Europe and North America (Mandak et al. 2004; Pyšek et al. 2003; Richards et al. 2008), more and more studies have reported later-generation hybridization, sexual reproduction, and phenotypic diversity (Bailey et al. 2007; Bram and McNair 2004; Forman and Kesseli 2003; Gammon et al. 2007; Grimsby et al. 2007; Mandak et al. 2003; Richards et al. 2008; Tiebre et al. 2007). I have observed extensive seed production throughout Long Island and seedling establishment at many sites (personal observation). Whatever barriers to sexual reproduction were originally present, such as the lack of male fertile plants (Hollingsworth et al. 1998), those barriers appear to have been overcome. Despite the evidence for sexual reproduction, clonal spread continues to be important for Japanese knotweed invasion on Long Island, as evidenced by the presence of a single, widespread *F. japonica* genotype in both roadside and salt marsh habitats (Richards et al. 2008). Significant among-population variation in a common garden for ecologically important traits such as lamina size, SLA, and growth rate, and habitat differentiation for SLA suggest that sexual reproduction and adaptive evolution may be contributing to Japanese knotweed's spread into beaches. Future studies involving reciprocal transplants and the measurement of correlations between traits and fitness in specific environments will help to clarify the importance of local adaptation in this system.

Phenotypic plasticity also seems to be playing an important role in Japanese knotweed invasion. Plasticity in traits like lamina size, root/shoot, and succulence may help Japanese knotweed to survive in habitats ranging from roadsides and riparian zones to salt marshes and beaches. Plasticity, coupled with sexual reproduction and the potential for adaptive evolution, could increase the already high rate of spread of Japanese knotweed in North America, particularly in coastal areas. Invasion of coastal ecosystems by nonnative plants can have significant impacts on ecosystem-level processes (Dukes and Mooney 2004; Grosholz et al. 2000; Levin et al. 2006; Windham 2001) and presents a serious challenge to managers of these systems. Information on the genetic and phenotypic makeup of invasive species can aid managers in assessing the threat of future spread and help them to develop appropriate control strategies.

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Appendix

Table 4 Statistical output for the multivariate test of the effects of habitat and site nested within habitat on leaf area and specific leaf area (SLA) of Japanese knotweed plants at six field sites on Long Island, NY, USA

MANOVA output					
Source of variation	Pillai's trace	<i>F</i>	Num. <i>df</i>	Den. <i>df</i>	<i>P</i> value
Habitat	0.299	0.64	2	3	0.587
<i>Site (habitat)</i>	0.501	26.74	8	640	<0.001

Italicized text is used to indicate significant *P* values

Table 5 Statistical output for the multivariate test of the effects of habitat and site nested within habitat on leaf area and specific leaf area (SLA) of Japanese knotweed plants at six field sites on Long Island, NY, USA

Standardized canonical coefficients			
Effect	Response variable	Canonical coefficient 1	Canonical coefficient 2
Habitat	Lamina area	0.088	–
	<i>SLA</i>	0.252	–
<i>Site (habitat)</i>	<i>Lamina area</i>	1.265	0.041
	SLA	–0.239	1.092

Italicized text is used to indicate the variable that had the greatest effect on the MANOVA outcome, based on canonical coefficient 1

Table 6 Statistical output for the univariate tests of the effects of habitat and site nested within habitat on leaf area and specific leaf area (SLA) of Japanese knotweed plants at six field sites on Long Island, NY, USA

Univariate ANOVA output					
Response variable	Source of variation	<i>df</i>	Mean square	<i>F</i>	<i>P</i>
Lamina Area	Habitat	1	21,718	0.45	0.538
	<i>Site (habitat)</i>	4	48,022	42.12	<0.001
	Error	320	1,140		
SLA	Habitat	1	18,501	1.35	0.309
	<i>Site (habitat)</i>	4	13,706	14.03	<0.001
	Error	320	976.7		

Italicized text is used to indicate significant *P* values

Table 7 Statistical output for the multivariate test of the effects of habitat and site nested within habitat on stem density, stem plus leaf mass, proportion stem, and the residuals of reproductive mass on date of harvest of Japanese knotweed plants at six field sites on Long Island, NY, USA

MANOVA output					
Source of variation	Pillai's trace	<i>F</i>	Num. <i>df</i>	Den. <i>df</i>	<i>P</i>
Habitat	0.9590	5.85	4	1	0.300
<i>Site (habitat)</i>	1.1486	2.42	16	96	0.004

Italicized text is used to indicate significant *P* values

Table 8 Statistical output for the multivariate test of the effects of habitat and site nested within habitat on stem density, stem plus leaf mass, proportion stem, and the residuals of reproductive mass on date of harvest of Japanese knotweed plants at six field sites on Long Island, NY, USA

Standardized canonical coefficients			
Effect	Response variable	Canonical coefficient 1	Canonical coefficient 2
Habitat	Stem density	–1.522	–
	Stem + lamina mass	1.195	–
	Reproductive mass residuals	0.329	–
<i>Site (habitat)</i>	<i>Proportion stem</i>	–2.162	–
	Stem density	0.375	0.797
	Stem + lamina mass	–2.218	–1.059
	<i>Reproductive mass resid.</i>	2.464	0.558
	Proportion stem	–0.635	–0.635

Italicized text is used to indicate the variable that had the greatest effect on the MANOVA outcome, based on canonical coefficient 1

Table 9 Statistical output for the univariate tests of the effects of habitat and site nested within habitat on stem density, stem plus leaf mass, proportion stem, and the residuals of reproductive mass on date of harvest of Japanese knotweed plants at six field sites on Long Island, NY, USA

Univariate ANOVA output					
Response variable	Source of variation	df	Mean square	F	P
Stem density	Habitat	1	2.4103	0.01	0.937
	Site (habitat)	4	341.37	2.35	0.083
	Error	24	145.15		
Stem + lamina mass	Habitat	1	544,695	7.85	<i>0.049</i>
	Site (habitat)	4	69,410	0.68	0.614
	Error	24	102,349		
Reproductive mass residuals	Habitat	1	1001.01	1.28	0.321
	Site (habitat)	4	782.30	1.93	0.137
	Error	24	404.53		
Proportion stem	Habitat	1	0.0364	6.42	0.064
	Site (habitat)	4	0.00566	2.20	0.099
	Error	24	0.00257		

Italicized text is used to indicate significant P values

Table 10 Statistical output for the multivariate test of the effects of drought treatment, habitat, and site nested within habitat on lamina area, specific leaf area (SLA), total dry mass, and root-to-shoot ratio for Japanese knotweed plants in the greenhouse

MANOVA output					
Source of variation	Pillai's trace	F	Num. df	Den. df	P
Habitat	0.9612	6.19	4	1	0.292
Site (habitat)	0.7328	5.16	16	368	<0.001
Treatment	0.9861	17.72	4	1	0.176
Treatment × site (habitat)	0.3284	2.06	16	368	<i>0.001</i>
Treatment × habitat	0.7778	0.88	4	1	0.655

Italicized text is used to indicate significant P values

Table 11 Statistical output for the multivariate test of the effects of drought treatment, habitat, and site nested within habitat on lamina area, specific leaf area (SLA), total dry mass, and root-to-shoot ratio for Japanese knotweed plants in the greenhouse

Standardized canonical coefficients			
Effect	Response variable	Canonical coefficient 1	Canonical coefficient 2
Habitat	Lamina area	-0.2337	-
	SLA	0.4971	-
	Total mass	-3.5998	-
	Root/shoot	-4.0761	-
Site(habitat)	<i>Lamina area</i>	<i>1.6519</i>	0.0334
	SLA	0.3481	-0.0643
	Total mass	0.4283	1.1433
	Root/shoot	0.6565	-0.2701
Treatment	Lamina area	0.3328	-
	SLA	-0.6519	-
	Total mass	0.7514	-
	Root/shoot	-1.9944	-
Treatment × site (habitat)	Lamina area	1.2646	-0.4199
	SLA	0.0147	1.2533
	Total mass	1.3214	1.6647
	Root/shoot	<i>1.3278</i>	0.1145
Treatment × habitat	<i>Leaf area</i>	<i>2.0743</i>	-
	SLA	1.0222	-
	Total mass	-1.7554	-
	Proportion stem	0.3212	-

Italicized text is used to indicate the variable that had the greatest effect on the MANOVA outcome, based on canonical coefficient 1

Table 12 Statistical output for the univariate test of the effects of drought treatment, habitat, and site nested within habitat on lamina area, specific leaf area (SLA), total dry mass, and root-to-shoot ratio for Japanese knotweed plants in the greenhouse

Univariate ANOVA output					
Response variable	Source of variation	df	Mean square	F	P
Lamina area	<i>Model</i>	11	5,173.0	14.86	<0.001
	Error	91	348.22		
	Habitat	1	179.11	0.51	0.475
	Site (habitat)	4	8,007.3	23.00	<0.001
	Treatment	1	16,090	46.21	<0.001
	Treatment × site (habitat)	4	799.31	2.30	0.065
SLA	<i>Treatment × habitat</i>	1	1,519.51135	4.36	<i>0.040</i>
	<i>Model</i>	11	6,683.4	2.53	<i>0.008</i>
	Error	91	2,646.5		
	Habitat	1	15,103	5.71	<i>0.019</i>

Table 12 (continued)

Univariate ANOVA output					
Response variable	Source of variation	df	Mean square	F	P
Total mass	<i>Site (habitat)</i>	4	8,050.8	3.04	<i>0.021</i>
	Treatment	1	4,701.4	1.78	0.186
	Treatment × site (habitat)	4	4,318.2	1.63	0.173
	Treatment × habitat	1	81.615	0.03	0.861
	<i>Model</i>	11	385.95	10.93	<i><0.001</i>
	Error	91	35.322		
	Habitat	1	74.107	2.10	0.151
	<i>Site (habitat)</i>	4	99.237	2.81	<i>0.030</i>
	<i>Treatment</i>	1	3257.0	92.21	<i><0.001</i>
	Treatment × site (habitat)	4	51.613	1.46	0.221
Root/shoot	Treatment × habitat	1	0.54741	0.02	0.901
	<i>Model</i>	11	0.65150	3.55	<i><0.001</i>
	Error	91	0.18369		
	Habitat	1	0.38772	2.11	0.150
	Site (habitat)	4	0.30026	1.63	0.172
	<i>Treatment</i>	1	4.9732	27.07	<i><0.001</i>
	Treatment × site (habitat)	4	0.11138	0.61	0.659
	Treatment × habitat	1	0.05852	0.32	0.574

Italicized text is used to indicate significant *P* values

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