Determining the role of natural selection in plant invasion: a study of introduced Japanese knotweed (*Fallopia japonica*) and a native relative, woodland knotweed (*Persicaria virginiana*)

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Abstract of the Dissertation

Determining the role of natural selection in plant invasion: a study of introduced Japanese knotweed (*Fallopia japonica*) and a native relative, woodland knotweed (*Persicaria virginiana*)

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The ecological and evolutionary study of plant invasion processes is of exceeding importance in today's changing environment. However, few studies have addressed the impact of natural selection on invasive plant species. While scientists have been able to detect selection in natural populations, most studies are not replicated in space or time leading to unreliable statistical estimates and tentative causal analyses. My objective was to further our knowledge of selection dynamics in the wild by working in the area of invasion biology through studies that combine both field and controlled settings. It has been suggested that plant invasion affords us the ability to better assess the speed and predictability of local adaptation by natural selection, and that there are at least two mechanisms by which species can become invasive: through rapid local adaptation and/or through augmented phenotypic plasticity. I conducted a three-year phenotypic selection analysis on invasive *Fallopia japonica* (Japanese knotweed) and a native relative, *Persicaria virginiana* (woodland knotweed) and have shown that natural selection is generally strong in these field populations and is highly variable, in both strength and direction, through time. To assess the level of local adaptation in *F. japonica* and compare it to *P. virginiana*, I conducted a reciprocal transplant experiment in the field using two contrasting light regimes. Neither species is particularly locally adapted to different light regimes and both show evidence of possessing traits that respond plastically to the different light conditions. I examined this phenotypic plasticity with a common garden experiment. Both species show plasticity for traits relating to increased fitness in the different light treatments (ambient, and 50% shade) that would aid either one in the establishment and invasion of a novel light habitat. However, they do this with different strategies. *P. virginiana* has a more robust response, having similar fitness in both treatments and so can be classified here as a Jack-of-all-trades. *F. japonica* had a more opportunistic response with increased fitness in the higher quality (light) treatment, making it a Master-of-some.

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I hereby state that permission has been granted by the University of Chicago Press to reproduce the article "Selection dynamics in native and introduced *Persicaria* species" (Chapter 1) which I wrote with Massimo Pigliucci (who directed and supervised the research) and was published in International Journal of Plant Sciences in June 2010.

Introduction

Invasive species have important and often costly impacts on agriculture, commerce, biodiversity, ecosystem functioning, and even human health (Cox 2004). The widely cited estimated cost of dealing with invasive species in the United States is $137.2 billion annually (Pimentel *et al.* 2000). Clearly, the study of these species is socially important, but there is also theoretical value to the study of invasive species. Biological invasions may be thought of as natural evolutionary experiments to study the effects of possibly novel and intense selection on species that are in the process of aggressively expanding their ranges and adapting to their new environments. It has been suggested that plant invasion affords us the ability to better assess the speed and predictability of local adaptation by natural selection (Ledger and Rice 2007), and that there are at least two, non-mutually exclusive, mechanisms by which species can become invasive: through rapid local adaptation and/or through high phenotypic plasticity (Parker *et al.* 2003, Sexton *et al.* 2002). To reach conclusions about either of these mechanisms, though, an understanding of how natural selection acts on natural populations of the invasive species is necessary. The goal of this dissertation is to determine how natural selection influences the invasion of Japanese knotweed (*Fallopia japonica*) through local adaptation and/or phenotypic plasticity by comparing it with a native, noninvasive relative woodland knotweed, *Persicaria virginiana*.

Few studies have addressed the impact of natural selection on invasive plant species (Lambrinos 2004). Natural selection has been a foundation of evolutionary theory since Charles Darwin published *On the Origin of Species*. Yet it has recently been argued that we still do not have a good grasp on the way natural selection operates and how strong it is in natural populations (Hendry 2005), let alone in populations of invasive species. Kingsolver *et al.* (2001) performed an extensive review of the literature on natural selection studies done on wild populations and found that, while detectable selection is fairly common, it is generally weak. Accordingly, the first questions addressed here (Chapters 1 and 2) are as follows: Can we detect natural selection in wild populations of related native and introduced invasive species? If so, is this selection strong enough to promote what can be called rapid evolution, a response we might expect when an organism expands its range and encounters potentially novel environmental factors?

Many have implicated rapid local adaptation as one way in which species become invasive (Parker *et al.* 2002, Cox 2004). Modeling has shown that there could be a wide range of variation in terms of invasion rate depending on heritability, growth rate, and stabilizing selection (García-Ramos and Rodríguez 2002). In one model, the speed of invasion increased with increased trait heritability and increased individual growth rate, and invasions were moderately accelerated by environmental homogeneity (García-Ramos and Rodríguez 2002). However, there are few empirical data to support this hypothesis of rapid evolution. Weinig's study (2005) is a notable exception. Her work with agricultural weed populations that have only existed for 10-50 years has demonstrated rapid evolutionary change. In this short time frame, she has demonstrated that a common North American weed, *Abutilon theophrasti*, can locally adapt to growing among crops, as well as to the shorter, human-modified growing season of agricultural fields (Weinig 2005). The standard experimental test for local adaptation is a reciprocal transplant (Parker *et al.* 2003), and this is the method employed in Chapter 3 where individuals from *F. japonica* and *P. virginiana* populations were reciprocally transplanted in light and shade natural field conditions. If the ability to locally adapt is one factor that contributes to a successful invasion, then we expect to find that *F. japonica* is more locally adapted than *P. virginiana*; that is, its fitness should be greater at home than at a transplant site. If their fitness is the same regardless of the habitat, then this suggests that the traits contributing to fitness are plastic and adjust from one site to another.

The final experiment in this dissertation (Chapter 4) concerns phenotypic plasticity, which has been proposed as a first step in the biological invasion process by allowing newly arrived plants a wider range of environmental tolerances (Sexton *et al.* 2002). After this step, shuffling of genetic variation combined with natural selection may lock some of these changes in place. It has been long proposed that an "ideal weed" is one that has a broad tolerance, exhibiting high fitness in a variety of environments (Baker 1965, 1974). Plasticity to light regime has been studied in detail for a group of annual weeds in the genus *Polygonum*, and it has been demonstrated that they may have wider ecological breadth due to plastic responses that allow a plant to be more successful in more environments (Sultan 2001). Using a common garden experiment with a light and a shade treatment, we examined plasticity for traits related to light acquisition in the two species and how this plasticity relates to the fitness of each species under different light regimes. Is *F. japonica* a "Jack-of-all-trades" having high fitness in a wide range of environmental conditions, or perhaps it is a "Master-of-some" in which it is better able to respond to good conditions with an increase in fitness compared to the response in *P. virginiana* (terminology *sensu* Richards *et al.* 2006)?

Taken together, these experiments not only give us insights into the specific nature of the Japanese knotweed invasion, but also contribute to our knowledge of fundamental concepts in evolutionary biology. Species invasions afford us the ability to examine natural selection, adaptation, and phenotypic plasticity in systems that often have a good historical record and short timeline. These circumstances make species invasions ideal for use as natural experiments to study evolution.

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SELECTION DYNAMICS IN NATIVE AND INTRODUCED *PERSICARIA* SPECIES

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Plant invasions represent natural experiments that allow us to both explore the dynamics of natural selection in the wild and examine the evolution of an invader on contemporary timescales. We conducted a study of 10 natural populations of two invasive species (*Persicaria lapathifolia* and *Persicaria cespitosa*) and one native species (*Persicaria pensylvanica*) to quantify the amount of natural selection acting on these species to compare the selection dynamics to which each is exposed. We also conducted a germination trial to compare the potential for invasion determined by germination rate. A Lande-Arnold-style multiple regression selection analysis was performed on five morphological traits (height, stem diameter, leaf number, leaf shape, and leaf area) using flower number as our fitness proxy. Most selection was indirect and caused by correlations with other traits under selection. However, there was significant direct selection for increased leaf number in both natives and invasives and for thicker stems in just the invasives. The germination test showed that not only do the invasive plants have a significantly higher germination rate (>60% compared with 3% for the noninvasives) but they also germinate significantly faster; both findings have large implications for the ability of these two invasive species to spread.

Keywords: natural selection, introduced plants, field populations, regression analysis.

Introduction

Natural selection has been one of the fundamental pillars of evolutionary theory ever since Darwin (1859). Despite this central role, it was not until Lande and Arnold's (1983) influential paper detailing a quantitative method for measuring phenotypic selection that tools to compare natural selection among different populations became widely available. Since then, a number of reviews, including those of Endler (1986) and Kingsolver et al. (2001), have compared the results of published selection studies to assess the strength of different types of natural selection. They established that selection can be commonly observed under field conditions, with thousands of published estimates of the magnitude of selection coefficients (Kingsolver et al. 2001). Though the magnitudes of these coefficients tend to be small, they appear to be large enough to cause evolutionary change on so-called ecological (Kingsolver and Pfennig 2007) or contemporary (Kinnison and Hendry 2001) timescales.

Field studies of selection in natural populations are an important first step in the understanding of selection dynamics. They can then lead to hypotheses about the causal pathways underlying selection in the wild (Wade and Kalisz 1990; Frank 1997; Pigliucci and Kaplan 2006). However, few field studies have addressed the specific issue of selection in introduced and invasive populations (Lambrinos 2004). Biological invasions may be thought of as natural evolutionary experiments that we can use to study the effects of possibly new and intense selection pressures on species that are in the process of aggressively expanding their range. Depending on the stage of an invasion, these pressures can be novel relative to the native habitat of the invader or novel relative to what the invasive population is experiencing in the areas where it has been established longer. Regardless, the presence of these potentially novel and intense selection pressures can lead to rapid evolutionary change in an invasive population.

There is a general sense that the capacity for rapid evolutionary change can be one part of what makes invasive species invade and become a challenge to control (see Stockwell et al. 2003; Maron et al. 2004; Facon et al. 2006), despite little data supporting this proposal. It has also been postulated that land managers that employ biological control would benefit from taking this capacity for rapid evolution into account (Müller-Schärer et al. 2004). Although the capacity for rapid evolution is certainly not unique to invasive species (for examples, see Bone and Farres 2001; Kinnison and Hendry 2001), relatively quick adaptive changes in invasive populations have been demonstrated in some instances (Stockwell et al. 2003). There is therefore much theoretical and practical value in studying the selection dynamics of invasive populations where they are naturally occurring, to gain insight into rapid organismal adaptation.

In this study, we examine the selection dynamics of populations of invasive and native smartweeds, which are in the genus *Persicaria* (*Polygonum* sensu lato). We compare populations of the invasive *Persicaria lapathifolia* and *Persicaria cespitosa* to populations of the native *Persicaria pensylvanica* in Long Island, New York. These species were chosen because of

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their close phylogenetic relationships; they are part of a monophyletic group of five species within the Polygonaceae, with *P. lapathifolia* and *P. pensylvanica* being sister taxa (Kim and Donoghue 2008). There is a relatively small number of studies in the invasion biology literature comparing natives and congeneric invasives, as distinct from the more common practice of using unrelated native plants that happen to live in the same area as a control (Muth and Pigliucci 2006). The benefit of a phylogenetically informed study is that differences traceable to the vagaries of lineage histories are minimized (Harvey and Purvis 1991), allowing one to highlight the effects of processes such as natural selection (see Brock and Galen 2005; Muth and Pigliucci 2006).

In addition to measuring selection in these natural populations, we also collected achenes (hereafter, seeds) to test for differences in germination rate, which are related to a plant's lifetime fitness (see Marks and Prince 1981; Sultan 2001). A high germination rate is often implicated in a plant's ability to invade. For instance, Mihulka and colleagues (2006) found that the ability to germinate in the light was the only predictor of invasion success of *Oenothera* in six European countries. Another study stressed the importance of prolific seedling emergence in helping a nonnative plant become naturalized in a new environment (Van Kleunen and Johnson 2007). However, despite frequent references in the literature to the importance of germination to a successful invasion, relatively little empirical work has been done on it.

We will address the following specific questions: (i) On what traits is selection acting in the native and invasive *Persicaria*? (ii) Are there differences in patterns of selection between invasives and natives? (iii) Are there differences in germination rates between the native and invasive populations that may be germane to the issues of invasion and differential fitness?

Given the dearth of studies on selection in natural populations of invasive plants, we cannot predict which specific traits will experience selection pressures in any given environment and genetic background. However, we predict that the two species classified as invasive (*P. cespitosa* and *P. lapathifolia*) should experience stronger selection or have more traits under selection due to their populations' expansion into new environments for which they might not be well suited. We further expect that the germination rates—a likely major component of fitness—in the invasives will be higher than the germination rate of the native species, illustrating a potentially important explanation for their invasiveness.

Material and Methods

Plant Species

The plants used in this study were annuals in the genus *Persicaria* (*Polygonum* sensu lato): *P. pensylvanica* (L.) M. Gómez, *P. lapathifolia* (L.) Gray, and *P. cespitosa* (Blume) Nakai. These are herbaceous plants that are generally considered weedy. *Persicaria pensylvanica* is a species native to North America and can grow in most soil types (Mitchell and Dean 1978). *Persicaria lapathifolia* has been introduced to eastern North America from Europe; it is listed as weedy or invasive according to several sources referenced by the USDA NRCS (2008) PLANTS Database. *Persicaria cespitosa* is also introduced, but its origins are in southeastern Asia (Mitchell and Dean 1978). It too is considered an invasive pest; in fact, its movement, sale, and cultivation are banned in the state of Connecticut (Connecticut Invasive Plants Council 2004), and it has a "significant threat" ranking in Tennessee (Southeast Exotic Pest Plant Council 1996). Long Island has other native *Persicaria* (such as *P. careyi* and *P. punctata*) but they could not be included due to extremely small population size observed in the field (often under 5 individuals).

Study Sites

All populations were located in Nassau and Suffolk counties of Long Island, New York (table 1). We had populations of at least one species at each of the following locations: Bethpage State Park (BP), Caleb Smith State Park (CS), Caumsett State Park (CM), Inlet Pond County Park (IP), the Nature Conservancy's Mashomack Preserve (MP), a farm in East Moriches (EM), and a yard waste dump behind Stony Brook University's baseball field (SB).

All measurements were taken between the end of summer and early fall of 2006, while the plants were flowering and setting seed. Table 1 features the coordinates for locating each population and the number of individuals in each population. Sampling was random within each population, with the exception of the population of *P. pensylvanica* at Bethpage State Park, for which we conducted a census of all individuals at the site.

Phenotypic Measurements and Selection Analysis

The following phenotypic traits were measured for each plant: leaf number, height, and stem diameter (taken at the tenth node). Additionally, we measured the length and width of one leaf from each plant, taken at the tenth node or the next node up that had a leaf. The length and width were mul-

Table 1

Site Information

Persicaria population	No. individuals	Coordinates
lap CM	100	40°55′1.61″N, 73°28′32.19″W
lap CS	100	40°51′19.87″N, 73°13′30.21″W
lap BP	50	40°45′16.04″N, 73°27′52.95″W
lap SB	50	40°55′22.52″N, 73 7′29.32″W
ces CS	100	40°51′24.04″N, 73°13′32.08″W
ces EM	37	40°47′27.50″N, 72°46′7.17″W
ces IP	40	41°6′32.26″N, 72°22′54.53″W
ces MP	40	41°3′15.15″N, 72°18′29.34″W
pen BP	23[a]	40°45′19.18″N, 73°28′2.58″W
pen SB	38	40°55′22.17″N, 73°7′28.98″W

Note. lap = *Persicaria lapathifolia*, ces = *Persicaria cespitosa*, pen = *Persicaria pensylvanica*. BP = Bethpage State Park, CS = Caleb Smith State Park, CM = Caumsett State Park, IP = Inlet Pond County Park, MP = Nature Conservancy's Mashomack Preserve, EM = a farm in East Moriches, and SB = a yard waste dump behind Stony Brook University's baseball field.

[a] This is not a sample but a census of every individual at the site.

tiplied to produce an estimate of leaf area, and the ratio of leaf length to leaf width was our estimate of leaf shape. These morphological traits were chosen due to their likely importance for a plant's fitness, as most are related to light acquisition, which affects photosynthesis. We used flower number as a fitness proxy; all three species produce one seed per flower.

We tested for species and population level differences using a nested ANOVA in JMP, version 7.0.1 (2007) using the unstandardized trait values. Species was the main factor and population of origin was the nested factor. All traits were normally distributed with the exception of leaf number, which was square root transformed to make it approach normality. We used JMP to conduct a principal components analysis (PCA) to examine how the plants were distributed in multivariate phenotypic space. The first two principal components were calculated using a correlation matrix obtained from the unstandardized data. We then plotted means and standard deviations for the principal components from each population to visualize the population means and degree of variation.

We used a Lande and Arnold (1983) selection analysis to estimate the strength and type of selection acting on the phenotypic traits in the three species. All analyses were performed in JMP. The dependent variable, relative fitness, was calculated by dividing the individual flower number by the mean flower number for its population. All trait measurements were standardized by dividing an individual's value by the standard deviation for that trait in that individual's population. The regression of individual traits on relative fitness produces standardized regression coefficients, which are interpreted as the selection differentials (s) that quantify the change of a trait mean caused by selection. This total selection includes the direct selection on that trait as well as indirect selection due to that trait's correlation with other characters. A multiple regression of all traits on relative fitness—accounting for intercharacter covariation—then produces the selection gradients (β), which estimate direct selection on individual traits while controlling for the influence of correlated traits (Lande and Arnold 1983).

Germination Protocols

A random sample comprising one-third of the plants measured at each location was fitted with a mesh bag over its top inflorescences to allow the seeds to complete their development. They were then collected at the end of the season (October 2006) and brought to the lab to be stored in a refrigerator at 6°C. Before the germination trial, we subjected the seeds to a cold stratification treatment: seeds were placed in small petri dishes over a piece of filter paper and covered with distilled water. The plates were sealed with Parafilm and kept in a 6°C refrigerator for 2 wk. The seeds were planted 0.5–1 cm under the surface of Sunshine Mix medium (Sun Gro Horticulture Canada, Edmonton, Alberta) in $3 \times 3 \times 5$-cm planters. We planted 50 seeds in six flats; each flat had 5 seeds from each site planted randomly. We watered and checked the seeds for germination daily for 1 mo. A contingency analysis was performed in JMP to determine whether the germination proportions were different using Pearson's χ^2 statistic. An ANOVA was run in JMP to examine differences in mean germination time.

Results

The nested ANOVA showed that differences due to species accounted for approximately one-third of the variation in leaf number, height, and stem diameter (table 2). The effect of species on leaf shape was the highest, explaining 71% of the variance in that trait. Within-population variation tended to be high in all traits, accounting for between 27% and 61% of the variation measured.

PCA was used to explore the relative position of the three study species in multivariate phenotypic space (fig. 1). We focused on principal components 1 and 2 because together they explained ~80% of the variation in the data (table 3). Principal component (PC) 1 was mostly influenced by variation in height, stem diameter, and leaf area (all weighing positively), while leaf number and leaf shape variation more heavily influenced PC 2 (the first positively and the second negatively, indicating an inverse relationship between these two variables). The invasive *Persicaria cespitosa* clustered in an area of multivariate space distinct from the other invasive, *Persicaria lapathifolia*, as well as from the native *Persicaria pensylvanica* (the latter two clearly overlapping), with the separation being evident along both principal components axes. Because variation in height, stem diameter, and leaf area define PC 1, we can say that most of the difference seen between the invasives (*P. cespitosa* and *P. lapathifolia*) is attributable to these traits.

Total selection favored larger plants in most populations—taller, leafier individuals with thicker stems and, to a lesser extent, larger leaves (the latter mostly in *P. lapathifolia*; table 4). We found virtually no selection (direct or indirect) on leaf shape in nearly all populations. These total selection values are selection differentials (s) from linear regressions, so they do not separate direct selection on a given trait from broader selection effects due to that trait's correlations with other characters. When we examined the direct selection coefficients (β), we found even fewer significant entries (table 4) and smaller values of the coefficients. The mean of the absolute values of significant selection gradients was 0.55, whereas the equivalent mean for total selection was 0.79. When sorted into native and invasive plants, both groups had half of their selection gradients reach significance. Direct selection on leaf number was significant for all populations except the CS and BP *P. lapathifolia*. Interestingly, mean leaf number for these two populations was higher than all others (fig. 2). Only about half of the selection gradients for height were significant, despite significant differentials in almost all cases for total selection on that trait, indicating that much of the selection on height was indirect. There were significant positive selection gradients for stem diameter in *P. cespitosa* at CS and in three populations of *P. lapathifolia* at SB, CS, and BP. The fourth *P. lapathifolia* population (CM) had a significant negative selection gradient, indicative of selection for a smaller stem diameter. Only three selection gradients for leaf shape were significant, and all three were negative (*P. cespitosa* at CS and *P. lapathifolia* and *P. pensylvanica* at SB). A negative gradient for leaf shape indicates selection for a less elongated and more square shape. Those same three populations had significant negative selection gradients for leaf area as well.

The selection gradients and corresponding population means for each trait are summarized in figure 2. Trait means were consistently similar for the two native *P. pensylvanica*

Table 2

ANOVA for Each Phenotypic Trait

Trait	SS	df	MS	*F* ratio	*P*	Variance component (%)
Square root leaf no.:						
Among species	1136.01	2	568.00	3.92	.072	26
Populations within species	1015.07	7	145.01	30.82	3.374×10^{-36}	26
Within populations	2667.81	567	4.71			48
Total	4818.88	576				
Height:						
Among species	283,721.08	2	141,860.53	4.33	.060	34
Populations within species	229,245.36	7	32,749.34	47.11	1.192×10^{-52}	30
Within populations	394,878.80	568	695.21			36
Total	907,845.24	577				
Stem diameter:						
Among species	512.48	2	256.24	5.05	.044	26
Populations within species	355.16	7	50.74	19.50	2.011×10^{-23}	18
Within populations	1467.27	564	2.60			55
Total	2334.91	573				
Leaf area:						
Among species	16,804.95	2	8402.48	1.78	.238	8
Populations within species	33,122.51	7	4731.79	27.92	7.070×10^{-33}	30
Within populations	92,691.76	547	169.45			61
Total	142,619.22	556				
Leaf shape:						
Among species	1079.90	2	539.95	89.24	1.044×10^{-05}	71
Populations within species	42.35	7	6.05	4.77	$3.208\ \mathrm{x}\ 10^{-05}$	2
Within populations	693.94	547	1.27			27
Total	1816.19	556				

Note. Species is the main factor, with population of origin nested within species.

populations (open circles in fig. 2), whereas the populations of the two introduced species were more variable in phenotype. For stem diameter and leaf area, *P. cespitosa* populations tended to have smaller selection differentials than most *P. pensylvanica* or *P. lapathifolia* regardless of their mean trait values. However, the selection gradients did not fall out into clusters in the same way as the differentials (not shown). There was no separation in these data between the native *P. pensylvanica* and the two invasives, *P. lapathifolia* and *P. cespitosa*.

Results from the germination experiment showed a germinability difference between species: 66% of *P. cespitosa*, 76% of *P. lapathifolia*, but only 3% of *P. pensylvanica* seeds germinated (table 5). A contingency analysis showed that there was indeed a significant difference between the proportions of seeds germinated across the three species (Pearson's $\chi^2 = 155.023$, df = 2, $P < 0.0001$). A second contingency analysis, carried out without *P. pensylvanica*, showed that there was also a significant difference in germination percentages between *P. cespitosa* and *P. lapathifolia* (Pearson's $\chi^2 = 5.004$, df = 1, $P = 0.0253$). The amount of time it took for seeds to germinate was significantly different across the three species ($P < 0.0001$) according to an ANOVA with species as the main factor (fig. 3).

Discussion

Introduced weedy species have been estimated to cause about $27.9 billion in agricultural losses every year (Pimentel et al. 2001), and yet our understanding of their evolutionary dynamics is only just beginning to emerge. Our results contribute to the as yet small number of studies that investigate natural selection in field populations of invasive plants. As a plant is introduced and becomes invasive, there is a good possibility that it will be exposed to novel conditions that could exert selection pressures on the plant that were not present in its native range. Indeed, it has been suggested that the ability to respond rapidly to these novel selection pressures is one way in which introduced plants may become invasive (Lee 2002). This response has been demonstrated for the agricultural weed *Abutilon theophrasti*, which in just 50 yr has adapted to the growing schedules of the farms whose fields it invades (Weinig 2005). Invasions represent an opportunity to examine the results of a natural experiment; since invasions take place despite human efforts to limit the phenomenon, it will be beneficial to take advantage of this opportunity to investigate all manner of issues, ranging from changes in community dynamics to changes in selection pressures exerted on both the invaders and sympatric natives. Our goal was to begin to develop a quantitative understanding of the selection pressures that these invasive plants experience in the field.

We found significant variation in trait means among species as well as among conspecific populations, presumably reflecting both genetic diversity and environmental heterogeneity. A noticeable result was the degree of variation for leaf shape among species, where the rhombic-shaped leaves of *Persicaria cespitosa* are one of this species' distinguishing features, while

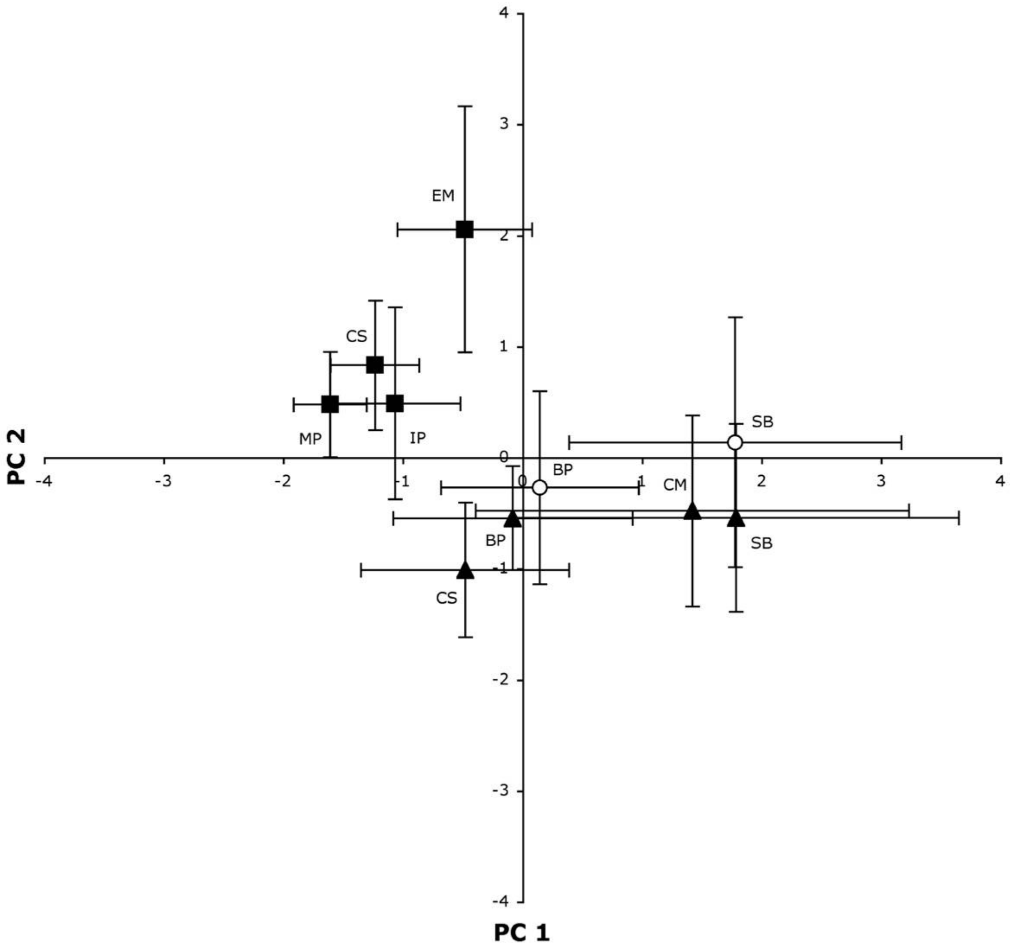

Fig. 1 Principal components (PC 1 and PC 2) for all traits in all populations (seem text for acronyms). The data points are based on the mean PC values for each population and the bars represent 1 SD. Squares = *Persicaria cespitosa*, triangles = *Persicaria lapathifolia* (invasives), and circles = *Persicaria pensylvanica* (native).

those of *Persicaria lapathifolia* and *Persicaria pensylvanica* are more elongate (Mitchell and Dean 1978). More generally, we found significant variation among populations within species for all traits measured. The variation in stem diameter was most likely due to the differences in growth habit among species: *P. cespitosa* is more prostrate and does not need the support of a thick stem as much as the more erect *P. pensylvanica* or *P. lapathifolia*. The PCA also separated *P. cespitosa*

Table 3

Eigenvalues and Eigenvectors from the Principal Components Analysis

Principal component	1	2	3	4	5
Eigenvalue	2.7258	1.2734	.5479	.2623	.1907
% variance explained	54.516	25.468	10.957	5.246	3.814
Eigenvectors:					
Square root leaf no.	.14368	.7654	.58324	.12959	.19121
Height	.55324	−.08863	−.10081	−.54474	.61576
Stem diameter	.55827	.10179	.04788	−.31614	−.75877
Leaf shape	.31526	−.61189	.63302	.35157	.04334
Leaf area	.51207	.14674	−.49665	.68038	.08163

Note. Underlining indicates traits weighing more on one or the other of the first two principal components.

Table 4

Total Selection (Selection Differentials, *s*) and Direct Selection (Selection Gradients, *β*) from the 10 Populations

Trait, population	Mean (unstd.)	Total selection (*s*)	*P*	Direct selection (*β*)	*P*
Leaf no.:					
ces CS	53.69	$\underline{.45}$	<.0001	$\underline{.33}$	<.0001
ces EM	140.73	$\underline{.83}$	<.0001	$\underline{.95}$	<.0001
ces MP	26.7	$\underline{.67}$	<.0001	$\underline{.38}$	.0117
ces IP	46.8	$\underline{.98}$	<.0001	$\underline{1.01}$	<.0001
lap SB	29.92	$\underline{1.00}$	<.0001	$\underline{.67}$	<.0001
lap CM	25.86	$\underline{1.60}$	<.0001	$\underline{1.91}$	<.0001
lap CS	13.5	$\underline{.65}$	<.0001	.08	.1763
lap BP	18.18	$\underline{1.10}$	<.0001	.06	.7698
pen SB	77.95	$\underline{.93}$	<.0001	$\underline{.95}$	<.0001
pen BP	41.13	$\underline{1.34}$	<.0001	$\underline{1.28}$	.0012
Height:					
ces CS	33.79 cm	$\underline{.38}$	<.0001	$\underline{.17}$	.0112
ces EM	59.07 cm	$\underline{.45}$	.0026	−.16	.1206
ces MP	36.48 cm	$\underline{.69}$	<.0001	$\underline{.46}$	.0024
ces IP	48.52 cm	$\underline{.55}$	.0018	−.01	.9454
lap SB	118.40 cm	$\underline{.86}$	<.0001	$\underline{.35}$	.0133
lap CM	111.80 cm	$\underline{1.06}$	<.0001	−.02	.8823
lap CS	65.40 cm	$\underline{.75}$	<.0001	.01	.9151
lap BP	57.14 cm	$\underline{1.09}$	<.0001	−.20	.3578
pen SB	87.08 cm	$\underline{.42}$	.0093	$\underline{.25}$	.0286
pen BP	64.37 cm	.55	.0876	−.19	.7348
Stem diameter:					
ces CS	2.08 mm	$\underline{.35}$	<.0001	$\underline{.22}$	.0011
ces EM	2.54 mm	.21	.1730	−.03	.7301
ces MP	1.41 mm	.01	.9375	−.12	.2777
ces IP	1.71 mm	$\underline{.40}$	.0271	−.09	.5243
lap SB	4.38 mm	$\underline{.95}$	<.0001	$\underline{.46}$	.0016
lap CM	4.45 mm	$\underline{1.22}$	<.0001	$\underline{-.48}$	.0079
lap CS	2.45 mm	$\underline{.81}$	<.0001	$\underline{.59}$	<.0001
lap BP	2.41 mm	$\underline{1.23}$	<.0001	$\underline{.82}$	.0006
pen SB	5.51 mm	$\underline{.55}$	.0004	.07	.5522
pen BP	3.99 mm	$\underline{.88}$	.0055	.22	.6728
Leaf shape:					
ces CS	3.13	−.09	.2290	$\underline{-.12}$	.0513
ces EM	2.76	−.03	.8656	.00	.9829
ces MP	2.73	−.01	.9357	.02	.8595
ces IP	3.34	.16	.3905	.14	.1797
lap SB	6.24	$\underline{-.47}$	.0059	$\underline{-.23}$	.0189
lap CM	5.63	−.29	.1219	−.06	.4693
lap CS	5.77	.07	.4745	−.03	.4092
lap BP	5.35	$\underline{-.39}$	.0460	.07	.4777
pen SB	6.77	−.04	.8236	$\underline{-.14}$	.0419
pen BP	5.87	−.20	.6276	−.03	.9190
Leaf area:					
ces CS	7.61 cm^2	.06	.4701	$\underline{-.14}$	.0219
ces EM	11.81 cm^2	.20	.2113	.01	.8769
ces MP	5.79 cm^2	.08	.6289	.10	.3677
ces IP	9.86 cm^2	$\underline{.36}$	.0503	.03	.8069
lap SB	31.58 cm^2	$\underline{.78}$	<.0001	$\underline{-.45}$	.0095
lap CM	24.45 cm^2	$\underline{1.18}$	<.0001	.18	.1560
lap CS	5.86 cm^2	$\underline{.72}$	<.0001	$\underline{.33}$	<.0001
lap BP	22.74 cm^2	$\underline{1.19}$	<.0001	$\underline{.65}$	.0013
pen SB	27.28 cm^2	$\underline{.41}$	.0121	$\underline{-.30}$	.0148
pen BP	6.67 cm^2	$\underline{.82}$	.0279	.20	.5145

Note. lap = *Persicaria lapathifolia*, ces = *Persicaria cespitosa*, pen = *Persicaria pensylvanica*. BP = Bethpage State Park, CS = Caleb Smith State Park, CM = Caumsett State Park, IP = Inlet Pond County Park, MP = Nature Conservancy's Mashomack Preserve, EM = a farm in East Moriches, and SB = a yard waste dump behind Stony Brook University's baseball field. Underlined *s* and *β* values are significant at the $P \leq 0.05$ level.

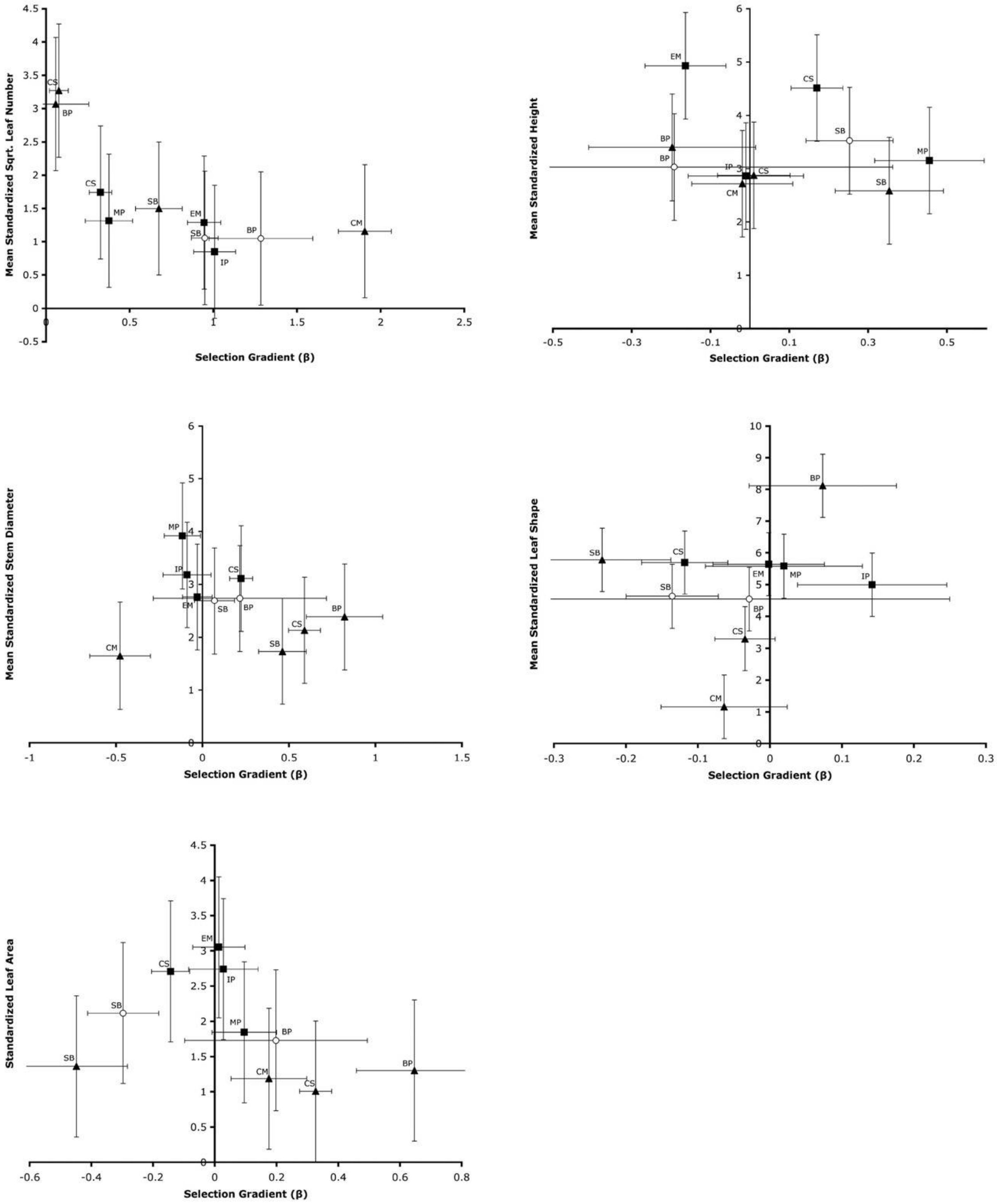

Fig. 2 Selection gradients from the selection analyses compared to the means of the corresponding trait. Horizontal bars associated with points are 1 SE (a measure of accuracy), and vertical bars are 1 SD (a measure of dispersal). Squares = *Persicaria cespitosa*, triangles = *Persicaria lapathifolia* (invasives), and circles = *Persicaria pensylvanica* (native).

Table 5

Results from the Germination Trial of the Three Species

Species	Seeds planted	Seeds germinated	Percent germination
Persicaria cespitosa	199	131	66
Persicaria lapathifolia	200	152	76
Persicaria pensylvanica	100	3	3

from the remaining two species in multivariate space, which is not too surprising, being that *P. lapathifolia* and *P. pensylvanica* are sister species (Kim and Donoghue 2008). It is interesting that the other invasive species, *P. lapathifolia*, did not occupy a PC space near *P. cespitosa*, raising the possibility that their invasiveness may be related to different combinations of phenotypic traits. Also, trait means were more variable in the invasive populations than the native ones, again a factor that may contribute to the invasiveness of *P. lapathifolia* and *P. cespitosa*.

Not surprisingly, the selection analyses showed that total selection generally favored larger plants (i.e., taller individuals, with more leaves and thicker stems). With some exceptions, this pattern held when we examined direct as opposed to total selection. These findings are in line with the findings of a recent review of natural selection estimates in the wild (Kingsolver and Pfennig 2007). Kingsolver and Pfennig compared the selection gradients reported in 42 studies that included morphological traits related and unrelated to body size. They found consistent directional selection for increased body size in agreement with Cope's rule, which states that organisms tend to evolve increased size over time (Hone and Benton 2005). In the case of the species under investigation in this study, larger size may have several obvious benefits given their ecology, including better access to light through overtopping of neighbors. Specifically, the populations of *P. lapathifolia* occurred mostly in densely packed communities, where light at ground level is low. In this instance, further manipulative experiments would bring to light any environmental causation that may be responsible for this pattern of selection, as a selection analysis by itself is not sufficient to elucidate causal pathways.

In general, our analyses revealed that much of the selection was indirect—a product of the correlations between traits. This finding was expected given that the morphological traits we measured are complex and are most likely the result of interacting genetic and developmental pathways. The mean of the absolute value of significant s was 0.79. When the selection due to correlation between traits was controlled for, we saw that the mean of β dropped to 0.55. Both positive and negative values of s and β occurred with equal frequency, so the mean of the absolute values is useful for comparison. Our mean of 0.55 falls near the "long tail" of strong selection described by Kingsolver et al. (2001), indicating that many of the populations studied are subject to relatively strong selection pressures in the wild. Further research should aim at untangling the factors that are causing this selection.

When comparing simply the numbers of significant selection gradients, the invasive populations and the native populations were the same (20 out of 40 gradients significant and 5 out of 10 gradients significant, respectively). Both groups also had very similar mean strengths of selection gradients. Based on these populations, there does not seem to be any difference between the natives and invasives in terms of the amount and force of selection that each faces under natural conditions.

Out of all 50 total pairings of differentials and gradients, only one (stem diameter at CM) had both estimates as significant but of opposite sign. This indicates that selection due to correlated traits may be counteracting direct selection on stem diameter. This is in agreement with the general finding by Kingsolver et al. (2001) that such pairs are relatively rare compared to those that are of matching sign, suggesting that the countering effects of correlated traits seldom reverse the pattern imposed by direct selection.

One of the common pitfalls of selection analyses is the problem posed by the possibility of unmeasured traits that nonetheless affect fitness dynamics (Mitchell-Olds and Shaw 1987). Our study, of course, is no exception, as it may well be that there are traits under selection in the three species that are correlated with the ones we measured and have an as yet undetected effect on fitness. The traits we used in this selection study were chosen because they were not highly correlated with each other based on preliminary analyses. This prescreening was necessary because multiple regression analyses are sensitive to covariance (Lande and Arnold 1983), but this reduced the number of potentially biologically interesting characters we could include in our analyses. If one or more of those missing traits has large effects on the fitness of an individual, then estimates of selection coefficients will be incorrect and any conclusion about causality generated from the study could be misleading. This is just one reason why selection studies are only the first step in understanding natural selection in wild populations. Manipulative experiments are necessary to examine specific causal pathways that may be suggested by

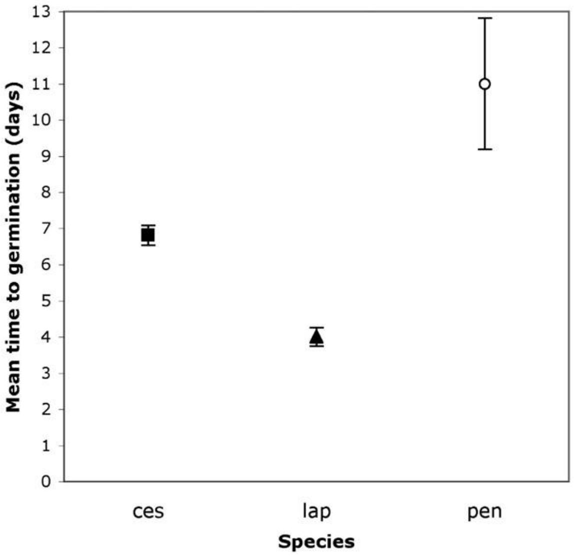

Fig. 3 Mean number of days until germination for the three study species. Bars represent 95% confidence intervals.

the results of the selection analysis (Mitchell-Olds and Shaw 1987, 1990; Pigliucci and Kaplan 2006).

Furthermore, selection analyses such as the one presented here are investigating the strength of selection, not specifically the response. Additional field seasons using the same populations would allow us to both observe whether the strength of selection changes over time and whether any differences in population change, which would then be interpreted as evidence of a response to the observed selection pressures. Another avenue for further study is to examine the patterns of quantitative genetic variation in each of these populations, which will affect the ability of the populations to respond to the observed selection pressures and can also have an effect on the progress of an invasion. For example, it has been shown recently that multiple introduction events increase the genetic variation of invasive reed canary grass (Lavergne and Molofsky 2007), leading to more opportunities for selection, which in turn may lead to rapid evolution of the invader. Similarly, there has also been great interest in the effect of gene flow of genes associated with domesticated traits from crop species into wild relatives—a process that is also likely to increase genetic variation and thereby affect responses to selection. There is concern that this type of gene flow, coupled with strong selection pressure, may have the potential to create highly invasive hybrid species in a relatively short period of time (for examples, see Barbour et al. 2006; Mercer et al. 2007).

In general, and despite much variation in trait means and selection coefficients, we found no clear pattern of differences separating native and invasive plants, although one of the invasives, *P. cespitosa*, inhabits a distinct area of multivariate phenotypic space. However, we did observe a staggering difference in their germination ability. The native *P. pensylvanica* was significantly slower to germinate and had much lower germination success compared to the two invasive species, *P. lapathifolia* and *P. cespitosa*. Due to the use of a single set of conditions during the germination trial, the differences between the native seeds and the invasive seeds could be due to differences in either dormancy or seed viability. However, even if that is the case, our findings still support the conclusion that the invasives' germination rates are an important part of their invasiveness, since seeds that are inviable or dormant for longer periods would hinder the potential spread of the natives. In the future, incorporating germination as a trait in selection studies of these species will allow us to assess the impact of this early aspect of life history on the invasion process.

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Chapter 2

Temporal variation in selection: a three-year field study of natural selection in invasive *Fallopia japonica* (Japanese knotweed) and native *Persicaria virginiana* (woodland knotweed)

Introduction

The ability of an invasive organism to respond to natural selection may be more important to its success than having broad physiological tolerances or possessing plastic traits (Lee 2002). However, few studies have addressed the impact of natural selection on invasive plant species (Lambrinos 2004, Novak 2007) and even fewer have monitored how the strength of selection may change over time in natural populations, invasive or otherwise (Siepielski *et al.* 2009). Natural selection has been a foundation of evolutionary theory since Charles Darwin published *On the Origin of Species*. Yet it has recently been argued that we still do not have a good grasp of the way natural selection operates in natural populations (Hendry 2005). This deficiency is curious considering how the ability to rapidly evolve is often implicated in what makes an invasive plant species invasive. Biological invasions may be thought of as natural (and sometimes indirectly human-induced) evolutionary experiments that scientists can use to study the effects of possibly novel and variable selection pressures on species that are in the process of aggressively expanding their range.

Kingsolver *et al.* (2001) conducted an extensive review of the literature on natural selection studies done in the field. They focused on those studies done in natural populations since Lande and Arnold (1983) proposed their method of measuring selection using multiple regression techniques. By sifting through thousands of estimates of selection gradients published between 1984 and 1997, Kingsolver and colleagues found that natural selection is indeed commonly observed in the field. However, despite this plethora of work, it is still questionable whether evolutionary biologists have a good sense of the strength of selection in natural populations (Hendry 2005). Most of the work reviewed by Kingsolver *et al.* (2001) was not replicated in space or time; in fact, the median number of temporal replicates was one. Siepielski *et al.* (2009) used criteria similar to the Kingsolver *et al.* (2001) in their review of selection analyses in the wild and found that out of 1,569 studies, only 89 (about 6%) had selection coefficients reported for two or more years. From this subset of 89 studies, they showed that the strength of selection can be highly variable and reversals in the direction of selection are common (Siepielski *et al.* 2009). In addition to these issues, a number of other questions regarding selection also remain largely unaddressed: How strong are specific types of selection (stabilizing, directional, and disruptive) in nature (Kingsolver and Pfennig 2007)? Can we predict evolutionary trajectories in natural populations (Postma *et al.* 2007)? What effect might temporally variable selection have on populations of invasive species?

In this study, our objective is to improve our knowledge of selection dynamics in wild populations of two related species, the introduced invasive *Fallopia japonica* (Japanese knotweed) and the native *Persicaria virginiana* (woodland knotweed). We posed the following questions: (i) On what traits is selection acting in populations of *P. virginiana* and *F. japonica*? (ii) Are there differences in the patterns of selection between the native species and the invasive one? (iii) Are selection gradients consistent (in strength and direction) across multiple field seasons and among multiple populations? The overall goal is to be able to compare the selection pressures experienced by the two species in both a qualitative and a quantitative manner. Are they of comparable strength and direction? Are they on the same or different traits?

We have focused on traits that we expect to influence fitness (e.g., height, leaf morphology) due to their impact on plant photosynthesis, predicting that the invading species is experiencing stronger selection given its aggressive range expansion into a novel environment. Given that *F. japonica* is a primary colonizer of volcanic slopes in its native range and that it has been noted to grow best in full sun (Beerling *et al.* 1994), we hypothesize that the wide variety of light regimes that this species experiences in its invasive range represent novel habitat that can influence selection. This analysis will give us a sense of the natural conditions under which these two species exist and currently evolve and is the first step in determining whether rapid local adaptation and/or phenotypic plasticity are contributing to the spread of Japanese knotweed.

Materials and Methods

Plant Species

Japanese knotweed, *Fallopia japonica* (Houtt.) Ronse Decr, is an erect, herbaceous perennial plant that overwinters as an underground rhizome. Although it has been most recently placed in the genus *Fallopia* (Ronse Decraene *et al*. 2000), Japanese knotweed has also been known as *Polygonum cuspidatum* (Siebold & Zucc.) Moldenke and *Reynoutria japonica* Houtt. and much published work still uses these other designations. *F. japonica* has been listed as one of New York State's top invaders (IPC 2008) and is listed as a prohibited or quarantined weed in nine other states (USDA 2010). *F. japonica* is native to Japan, China, parts of Korea, and parts of Taiwan, and is currently a widespread pest in much of Europe and North America (Beerling *et al.* 1994). It was introduced to North America from Europe in the late 1800s after having been introduced there in the mid-1800s as an ornamental. It was initially heralded for its gracefully arched stems and clouds of tiny flowers, but it quickly fell out of favor as gardeners realized how difficult it was to remove from their gardens once established (Townsend 1997). It has become a noxious weed across Europe and North America, forming dense stands with a substantial canopy. The previous years' litter does not break down quickly, resulting in a thick covering of old stems on the ground, which is thought to prevent native plants from growing with them (Beerling *et al.* 1994). The hybrid, *F. x bohemica*, a plant of similar habit and morphology to Japanese knotweed, was shown to attain competitive superiority by limiting other species' access to light under its stands (Siemens and Blossey 2007).

Woodland knotweed, *Persicaria virginiana* (L.) Gaertn., is an erect, herbaceous perennial that grows from an underground rhizome, just as Japanese knotweed does. However, *P. virginiana* does not attain the size of *F. japonica* and is not listed as a noxious weed. *P. virginiana* is native to North America, where it is also known by another common name, jumpseed. This species was chosen as a comparison for *F. japonica* due to its relatedness and similar habit. A phylogenetically-informed comparison of species has the advantage of minimizing effects of differing evolutionary histories, allowing for differences caused by natural selection to be more easily interpreted (see Harvey and Purvis 1995; Brock and Galen 2005; Muth and Pigliucci 2006). However, the only other member of the genus *Fallopia* that is native to North America is not found in large populations; in fact, it is listed as rare or endangered in three states (USDA 2010). To find a related native species for comparison, we expanded our search to include all former members of the genus *Polygonum*, which at one time included both knotweeds (Japanese as *P. cuspidatum* and woodland as *P. virginianum*). A recent treatment using chloroplast DNA sequences has placed *Fallopia* as a sister group to the two clades that contain the *Persicaria* (Lamb Frye and Kron 2003).

Study Sites

All populations were located in Nassau and Suffolk Counties on Long Island, NY (Table 1). Populations of *P. virginiana* were located at the following sites: Avalon Gardens (AV), Bethpage State Park (BP), Caumsett State Park (CM), Caleb Smith State Park (CS), Hempstead Lake State Park (HL), Inlet Pond County Park (IP), and The Nature Conservancy's David Weld Preserve (DW) and Uplands Preserve (UP). Populations of *F. japonica* were located at the following sites: Caumsett State Park (CM), Lake Ronkonkoma County Park (LR), Mill Pond at Frank Melville Memorial Park (MP), Nissequogue River State Park (NR), Paul T. Given County Park (PG), and Stony Brook University's baseball field (SB). Four of the *P. virginiana* populations (BP, CM, CS, and HL) were part of a pilot selection survey in 2006 and data from that field season has been included in this study. The rest of the populations were measured in the 2007, 2008, and 2009 field seasons, with the exceptions of the *F. japonica* population at NR, which was destroyed by park staff in 2008, and the *P. virginiana* populations at CM, IP, and UP in 2008 due to early die off. Canopy cover at the study sites is variable (see Table 2), with mean photosynthetically active radiation at each site ranging from approximately 24 – 722 μmol s^{-1} m^{-2} (measured between 11 am and 1 pm on clear days with a LI-COR DataLogger (Model LI-1000) and terrestrial quantum sensor (Model LI-190), LI-COR Biosciences, Nebraska).

Phenotypic Measurements

All measurements were taken during the late summer/early fall of the year when the plants have flowered and set seed. *P. virginiana* measurements were done on entire individuals after carefully removing the shallow rhizomes from the ground to determine which stems belong to which individual plants; they were then replaced in the ground. *F. japonica*'s size and extensive, deep rhizome network prevented us from following the same procedure. Measurements of this species were done on three randomly chosen stems coming from the same crown and averaged. We measured the following phenotypic traits for each individual plant in each population: leaf number, height, leaf thickness, leaf length and leaf width. The leaf measurements were taken at the 10th node leaf or the next leaf up after that if there was no leaf at the 10th node. Leaf length and leaf width were multiplied to produce an estimate of leaf area and the ratio of leaf length to leaf width was used as an estimate of leaf shape. In addition to these morphological traits, we also measured F_v/F_m using an OS-30p Chlorophyll Fluorometer (Opti-Sciences, Inc., New Hampshire). F_v/F_m is a physiological measurement of efficiency proportional to the maximum quantum yield of the photosynthetic apparatus (Krause and Weis 1991). F_v is the difference between the constant or initial fluorescence (the state after a dark period when all photosynthetic reaction centers are open) and the maximum fluorescence (F_m) in light (Krause and Weis 1984). All traits chosen were likely to be important to a plant's fitness as they all related to light acquisition and photosynthesis. Additional morphological traits were measured (stem diameter, for example) but were removed from the analysis due to high correlation with other traits. We used number of inflorescences as the fitness proxy for both species.

For the 2007 field season, *F. japonica* seeds were collected from all six populations and nearly all individuals (100 total) and a randomized germination trial was performed. Seeds were cold stratified in a 6°C refrigerator in Petri dishes covered with a piece of filter paper and distilled water. Approximately 40 seeds per individual were planted 5 mm deep in Sunshine Mix 1 medium (Sun Gro Horticulture Canada, Ltd.) in 2x2x5 cm cell planters in the greenhouse at Stony Brook University. We watered and checked the seeds for germination daily for one month. Time to germination and total percent germination were recorded. *P. virginiana* seeds were also planted and treated in this manner with the additional acid scarification (required from previous experience), however, there was no germination recorded.

Data Analysis

All statistical analysis was performed using JMP 7.0.1 software (SAS Institute Inc., North Carolina). Basic statistics (trait means, standard deviations, etc.) were calculated from the raw data for all populations and years. Before further analysis, several traits needed to be transformed to meet the assumptions of the statistical analyses: in *P. virginiana*, leaf number, shape, and thickness were log normal transformed and leaf area was square root transformed; in *F. japonica*, leaf number was square root transformed and percent germination was arcsine transformed. We tested for year and population level differences for each species using a nested analysis of variance of the transformed trait values. We did a separate nested ANOVA for each species as their morphological traits are clearly different and so the species effect would naturally be significant in most traits. Population of origin was the main factor and year of measurement was the nested factor. A one-way ANOVA was done on the *F. japonica* germination data from 2007. We conducted a principal components analysis (PCA) of each species to examine how the plants and populations were distributed in multivariate phenotypic space. The principal components were calculated using a correlation matrix obtained from the transformed data.

We used a phenotypic selection analysis (Lande and Arnold 1983) to estimate the strength and direction of selection acting on the phenotypic traits in *F. japonica* and *P. virginiana*. Relative fitness (the dependent variable) was calculated by dividing each individual's inflorescence number by the mean inflorescence number for its population. As described by Lande and Arnold (1983), all trait measurements were standardized by dividing an individual's value by the standard deviation for that trait in that individual's population. The purpose of standardizing the traits in this fashion is to have the resulting selection coefficients in units of standard deviations. Regression of individual traits on relative fitness produces standardized regression coefficients, which are interpreted as the selection differentials (s) that quantify the total change of a trait mean caused by selection. The selection differential includes the selection directly acting on the trait of interest plus the selection due to that trait's correlations with other traits. The second step in the analysis is a multiple regression of all traits on relative fitness, which takes into account covariation among traits and produces the selection gradients (*β*). These gradients represent direct selection on individual traits because the multiple regression controls for the influence of correlated traits (Lande and Arnold 1983). The addition of a quadratic term to the regression model would allow us to look for evidence of stabilizing or disruptive selection, however in order to have the power to detect any significant effect of these types of selection we would have needed sample sizes much larger than the number of individuals found in each population. As a result of this constraint, we did not estimate quadratic selection coefficients.

To quantify overall patterns of temporal variation, we followed the methods outlined by Siepielski *et al.* (2009). We calculated standard deviations among selection coefficients (differentials and gradients) for each species in the following manner:

$$SD = \sqrt{\frac{1}{n-1}\sum_{t=1}^{n}(s_t - \bar{s})^2},$$

where *s* is the selection coefficient for a trait, *t* is the year, *n* is the number of years of the study. The output of this equation is in units of standard deviations, which is useful because the standardized selection gradients and differentials are also in these units. The above equation gives a measure of temporal variation due to both differences in strength and direction; to examine the variation in strength alone, we used the absolute values of the coefficients in a second set of analyses using the same equation (Siepielski *et al.* 2009).

Results

In *P. virginiana*, most of the trait variation was explained by variation among the years within each population (Table 3 and Figure 1). A significant component of the variance was explained by the different populations for only height (P = 0.0005). The *F. japonica* ANOVA displayed the same pattern, where variation within populations among years was significant for all traits and height was the only trait that differed among populations (Table 4 and Figure 1). For the germination trial of seeds from the 2007 *F. japonica* populations, there was no difference between the populations in percent germination (Table 5), which was high for all of them (80-95%). There was, however, a significant difference ($P < 0.0001$) between the populations in the time it took seeds to germinate.

We used principal components analyses on each species to examine the multivariate relationships between the traits used in the selection analysis. The eigenvectors for each principal component (Table 6) illustrate the relative independence of each trait. With the exception of PC2 for Japanese knotweed, each principal component was dominated by the influence of only one of the traits, suggesting independence of the traits measured.

In general, total selection (s) favored taller and leafier plants with larger leaves in *P. virginiana* and taller, leafier plants in *F. japonica* (Tables 7 and 8). However, an examination of the number of selection gradients (β) for those traits shows that direct selection favored taller, leafier *P. virginiana* and just leafier *F. japonica.* In total, 75 out of 142 selection differentials were significant ($P \leq 0.05$) for *P. virginiana* and 29 out of 90 were significant for *F. japonica*. There were approximately half as many significant gradients for each species (36 for *P. virginiana* and 15 for *F. japonica*). Most of the significant differentials and gradients were positive. The means of the absolute value of significant differentials and gradients were of similar strength in both species: 0.65 and 0.70, respectively, for *P. virginiana* and 0.47 and 0.46, respectively, for *F. japonica.*

To visualize the temporal variation in selection on specific traits, we graphed gradients for each of the six traits of interest for each population, separated by year of measurement for both species (Figures 2 and 3, different colors and symbols represent different source populations). Some traits, such as height and leaf number for *F. japonica*, had consistently positive (or no different from zero) selection gradients. Leaf thickness in both species had populations with significant positive and negative gradients. There were seven instances of reversals in direction of the selection gradients in a single population across the years of the study (in *F. japonica*, leaf area at SB and CM and in *P. virginiana*, height and leaf number at UP, F_v/F_m at DW and HL, leaf area at CM, and leaf thickness at UP).

We did a standard deviation analysis following the methods of Siepielski *et al.* (2009) to gauge the overall temporal variation in selection coefficients produced by our entire study. The distributions of SD for total variation in differentials and gradients were similar – right skewed with medians of 0.22 and 0.17, respectively (Figure 4, top). We used the absolute values of the coefficients in the same type of analysis to examine just the variation in strength of selection across years studied. Again, we saw right skewed distributions of SD for both differentials and gradients, with similar medians of 0.19 and 0.14, respectively (Figure 4, bottom).

Discussion

Analysis of the natural experiments represented by plant invasions contributes to a variety of different important topics in biology, including ecosystem ecology and restoration (for example, Yelenik and Levine 2010) and the response to global climate change (for example, Thomas 2010). Studying plant invasions also contributes to well-established tenets of biology, such as evolution and natural selection, by presenting an opportunity to examine what happens when a species aggressively expands its range into new environments that may be exerting novel selection pressures. Quantifying natural selection in the wild is a continuing aim of evolutionary biologists (Kingsolver *et al.* 2001, Hendry 2005). Given that invasive plants cause an estimated $27.9 billion in agricultural losses every year (Pimentel *et al.* 2001), it also behooves scientists to study plant invasions not only for their intrinsically fascinating dynamics, but also for their practical importance. The goal of our study was to shed light on the selection dynamics occurring in an invasive plant population's range. We also wanted to compare these dynamics with those of populations of a related, native, and non-invasive plant.

We found significant variation among years within populations of both *F. japonica* and *P. virginiana*, that most likely reflected genetic diversity and environmental heterogeneity. For *F. japonica* on Long Island, environmental heterogeneity is probably more important in creating these trait differences due to the lack of genetic variation in the Japanese knotweed populations (Richards *et al.* 2008). The only trait that was different among populations was height in both species, which we had predicted based upon observations in the field.

The six traits chosen for the phenotypic selection analysis were found to be largely independent of each other, as seen from the results of the PCA. The problem of trait correlation was initially discussed by Lande and Arnold (1983) in their description of quantifying natural selection because multiple regressions are sensitive to collinearity. Not only were the traits we used largely independent of one another (PC2 was the only component to have more than one trait weighing heavily on it), but they all also had the potential to have large fitness effects as they can all be related to light acquisition. Of course, a common challenge with phenotypic selection analyses is the possibility of unmeasured traits that nonetheless affect fitness (Mitchell-Olds and Shaw 1987). It may be that there are traits under selection in our study that are correlated with the ones we measured and therefore have an undetected effect on fitness. For this and other reasons, phenotypic selection analyses are only the first steps in understanding selection dynamics in the wild and should be followed by manipulative experiments to untangle the causal structure connecting fitness with an organism's traits (Pigliucci and Kaplan 2006).

Most of the significant selection coefficients were gradients, not differentials, indicating that most of the selection on the six traits of interest was indirect and due to correlations between traits. Being that most of our traits were not highly correlated with each other, this is further evidence for unmeasured correlated traits affecting fitness, as mentioned above. There could be one (or more) unmeasured traits that connect two measured traits, so their relationship is mediated by correlations that we cannot see with this analysis. Morphological traits are, generally, complex and arise through the interaction of several genetic and developmental pathways, so it is not surprising that most of the selection we found is correlational. The mean strength of significant selection gradients was 0.70 for *F. japonica* and 0.46 for *P. virginiana.* Both of these values would be characterized as part of the long tail of strong selection that Kingsolver *et al.* (2001) described in their review. Invasive *F. japonica* populations might be experiencing greater average selection because they are growing in novel environments outside of the species' native range where it is a primary colonizer of volcanic slopes (Beerling *et al.* 1994).

The significant selection that was present was mostly for larger plants and this result is consistent with a recent review of selection on morphological traits related and unrelated to body size. Kingsolver and Pfennig (2007) found consistent directional selection for an increase in body size regardless of what aspect of fitness the increase in size contributes to (fecundity, mating success, or survival). In terms of *F. japonica* and *P. virginiana*, larger size has clear benefits to an individual, such as the ability to successfully compete with neighbors for light. However, plant size is a complex morphological trait that can be influenced by factors both internal and external to the individual plant. Manipulative experiments with different light regimes would allow us to elucidate some of the causal pathways involved in the relationship between size and fitness.

We found much year-to-year variation in both the strength and direction of selection gradients across the three years of this study, with a small number of differences around 1 SD. These results are consistent with the findings of Siepielski *et al.* (2009), who observed a wide range of variation in strength and total variation among years of a given study. Indeed, they suggest that perhaps the common question of whether selection is strong or weak in nature would be better put in a context of annual variation in selection strength and how populations respond to this fluctuation of both the magnitude and direction of selection (Siepielski *et al.* 2009). A large amount of information about trait values and relative fitness through time is needed to be able to predict how a population may respond to this type of fluctuating selection. The work of the Grants in the Galapagos is a notable (and rare) example of this type of thorough study. In 2002, they published the results of a thirty-year analysis on selection that contained pedigree data, environmental conditions, and selection estimates. They were able to show great variation in selection over the years and even some patterns, such as some size traits being strongly selected every five years (Grant and Grant 2002). However, even with the large time span and vast amounts of data, there were still changes in the finch populations that could not have been predicted at the beginning of their experiment and as such, they stress the need for more long term studies that catch rare occurrences that may briefly have a huge impact on the selection regime (Grant and Grant 2002).

In populations that experience reversals in the direction of selection (such as the seven instances in six of the populations in this study), we should have slower (or no) evolution in the traits. Variation in the strength and direction of selection has the potential to limit phenotypic diversification in these natural populations. Given that most phenotypic selection analyses in nature are only carried out for one year (Siepielski *et al.* 2009), we believe our study contributes to the field by adding to the number of temporally replicated studies. Our study shows that the time frame of investigation can have a great effect on the outcome; if we had just looked at selection for a single field season, we would have missed the larger picture of variation. Temporal fluctuations in selection strength and direction have important implications for investigations of rapid or so-called contemporary evolution and temporally replicated studies are a must to shed light on this topic.

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Table 1: Population abbreviations and locations. All locations are in the state of New York, on Long Island.

Abbreviation	*Location*	*Species*	*Seasons*	*Coordinates*
AV	Avalon Gardens, Stony Brook	*P. virginiana*	’07, ’08, ’09	N 40° 54.7’ W 73° 9.1’
BP	Bethpage State Park, Bethpage	*P. virginiana*	’06, ’07, ’08, ’09	N 40° 45.1’ W 73° 28.1’
CM	Caumsett State Park, Lloyd Neck	*P. virginiana* *F. japonica*	’06, ’07, ’09 ’07, ’08, ’09	N 40° 55.0’ W 73° 28.6’ N 40° 56.2’ W 73° 27.3’
CS	Caleb Smith State Park, Smithtown	*P. virginiana*	’06, ’07, ’08, ’09	N 40° 51.3’ W 73° 13.5’
DW	David Weld Preserve, Nissequogue	*P. virginiana*	’07, ’08, ’09	N 40° 54.6’ W 73° 12.6’
HL	Hempstead Lake State Park, Rockville Centre	*P. virginiana*	’06, ’07, ’08, ’09	N 40° 40.9’ W 73° 38.8’
IP	Inlet Pond County Park, Greenport	*P. virginiana*	’07, ’09	N 41° 6.5’ W 72° 22.8’
LR	Lake Ronkonkoma County Park, Lake Ronkonkoma	*F. japonica*	’07, ’08, ’09	N 40° 56.2’ W 73° 7.0’
MP	Mill Pond at Frank Melville Memorial Park, Setauket	*F. japonica*	’07, ’08, ’09	N 40° 56.6’ W 73° 6.9’
NR	Nissequogue River State Park, Kings Park	*F. japonica*	’07	N 40° 54.0’ W 73° 13.7’
PG	Paul T. Given County Park, Smithtown	*F. japonica*	’07, ’08, ’09	N 40° 51.5’ W 73° 12.7’
SB	Baseball Field, Stony Brook University Campus	*F. japonica*	’07, ’08, ’09	N 40° 55.4’ W 73° 7.4’
UP	Uplands Preserve, Cold Spring Harbor	*P. virginiana*	’07, ‘09	N 40° 51.3’ W 73° 27.2’

Table 2: Mean photosynthetically active radiation at each population. *P. virginiana* populations measured with a quantum sensor at 1 m, *F. japonica* populations measured at 2 m.

Location	Average PAR (μmol s^{-1} m^{-2})
F. japonica	
CM	612.20
LR	264.79
NR	153.50
MP	99.56
PG	560.99
SB	27.36
P. virginiana	
AV	36.78
BP	24.09
CM	721.96
CS	55.98
DW	203.82
HL	63.99
IP	50.87
UP	27.43

Table 3: Nested analysis of variance for *P. virginiana* traits. Population of origin is the main factor with years nested within populations (2006-2009).

LN Leaf Number	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	56.1694	7	8.0242	2.2870	0.0775	3.60
Years w/i Pops	59.6452	17	3.5085	5.6232	< 0.0001	9.45
Within Years	715.0416	1146	0.6239			86.95
Total	830.8563	1170				100.00

Height	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	303722.6553	7	43388.9508	7.0568	0.000492	22.10
Years w/i Pops	104524.4679	17	6148.4981	8.0195	< 0.0001	11.07
Within Years	873261.3410	1139	766.6913			66.84
Total	1281508.4642	1163				100.00

SQRT Leaf Area	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	610.7944	7	87.2563	1.7366	0.1667	2.52
Years w/i Pops	854.1921	17	50.2466	9.2112	< 0.0001	16.27
Within Years	6033.1694	1106	5.4549			81.21
Total	7498.1559	1130				100.00

LN Leaf Shape	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	1.3910	7	0.1987	1.0255	0.4510	0
Years w/i Pops	3.1003	16	0.1938	3.3562	< 0.0001	5.27
Within Years	63.2199	1095	0.0577			94.73
Total	67.7112	1118				100.00

Fv/Fm	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	3.8136	7	0.5448	0.6691	0.6953	0
Years w/i Pops	13.0277	16	0.8142	31.6351	< 0.0001	46.50
Within Years	24.5027	952	0.0257			53.50
Total	41.3440	975				100.00

LN Leaf Thickness	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	6.0224	7	0.8603	0.4811	0.8319	0
Years w/i Pops	23.2480	13	1.7883	21.0966	< 0.0001	33.69
Within Years	78.4951	926	0.0848			66.31
Total	107.7655	946				100.00

Table 4: Nested analysis of variance for *F. japonica* traits. Population of origin is the main factor with years nested within populations (2007-2009).

SQRT Leaf Number	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	62.9127	5	12.5825	0.5429	0.7406	0
Years w/i Pops	231.7815	10	23.1781	5.5885	< 0.0001	18.79
Within Years	1256.6953	303	4.1475			81.21
Total	1551.3895	318				100.00

Height	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	391904.9344	5	78380.9869	11.5417	0.000677	38.95
Years w/i Pops	67911.2898	10	6791.1290	3.4905	0.000228	6.84
Within Years	587579.1108	302	1945.6262			54.21
Total	1047395.3350	317				100.00

Leaf Area	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	43813.3087	5	8762.6617	0.7903	0.5800	0
Years w/i Pops	110875.7106	10	11087.5711	8.9188	< 0.0001	28.62
Within Years	375434.8772	302	1243.1618			71.38
Total	530123.8965	317				100.00

Leaf Shape	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	0.8143	5	0.1629	1.7657	0.2077	5.28
Years w/i Pops	0.9223	10	0.0922	4.3491	< 0.0001	13.73
Within Years	6.4045	302	0.0212			80.98
Total	8.1411	317				100.00

Fv/Fm	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	0.7993	5	0.1599	0.5815	0.7143	0
Years w/i Pops	2.7494	10	0.2749	16.0003	< 0.0001	43.16
Within Years	5.1893	302	0.0172			56.84
Total	8.7380	317				100.00

Leaf Thickness	*SS*	*df*	*MS*	*F ratio*	*P*	*Variance Component*
Among Pops	0.1567	5	0.0313	1.7165	0.2184	7.47
Years w/i Pops	0.1825	10	0.0183	7.6901	< 0.0001	23.41
Within Years	0.7169	302	0.002374			69.12
Total	1.0561	317				100.00

Table 5: Analysis of variance for germination test of knotweed seeds from 2007 field season.

ARCSIN Percent Germination

	SS	*df*	*MS*	*F ratio*	*P*
Populations	0.5274975	5	0.105499	1.8546	0.1096
Error	5.4042014	95	0.056886		
Total	5.9316988	100			

Time to Germination

	SS	*df*	*MS*	*F ratio*	*P*
Populations	6.372987	5	1.2746	12.9614	< 0.0001
Error	9.145397	93	0.09834		
Total	15.518384	98			

Table 6: Principal components analysis. Bold indicates which of the principal components a given trait influences most.

P. virginiana

	Principal Component	*1*	*2*	*3*	*4*	*5*	*6*
	Eigenvalue	1.7286	1.2191	1.0085	0.9795	0.6164	0.4480
	% Variance Explained	28.809	20.318	16.808	16.325	10.247	7.466
Eigenvectors	*Height*	0.59141	-0.33333	0.14195	-0.05944	0.17686	**0.69582**
	Fv/Fm	-0.19307	**0.74972**	0.25071	0.03941	0.45465	0.35991
	LN Leaf #	0.4354	0.41191	0.40594	0.13674	**-0.67227**	-0.07299
	LN Leaf Thickness	0.19935	0.02843	-0.28926	**0.91912**	0.16537	-0.06033
	SQRT Leaf Area	**0.59646**	0.16202	-0.03048	-0.26652	0.47446	-0.56649
	LN Leaf Shape	-0.16699	-0.36067	**0.81707**	0.24575	0.24001	-0.23755

F. japonica

	Principal Component	*1*	*2*	*3*	*4*	*5*	*6*
	Eigenvalue	1.5784	1.4004	0.9603	0.8119	0.6818	0.5672
	% Variance Explained	26.306	23.341	16.005	13.531	11.364	9.453
Eigenvectors	*Height*	**0.5735**	0.10633	-0.4716	0.0947	0.10147	0.64662
	Leaf Thickness	-0.5374	0.31245	-0.00716	-0.08106	**0.7101**	0.32047
	Leaf Area	0.44064	0.21875	0.34849	**-0.76863**	0.19383	-0.09046
	Leaf Shape	0.28558	-0.38128	**0.65766**	0.42474	0.36228	0.17001
	Fv/Fm	-0.09892	**0.60485**	0.46363	0.15733	-0.48997	0.38025
	SQRT leaf #	0.31111	**0.57619**	-0.09289	0.43419	0.2767	-0.54544

Table 7: Total selection (selection differentials, s) and direct selection (selection gradients, β) from the *P. virginiana* populations. Bold s and β values are significant at the p≤0.05 level. Trait means were calculated from the unstandardized data.

Trait	Population & Year	Trait Mean	Total selection (s)	P	Direct Selection (β)	P
Fv/Fm	AV07	0.63	0.10	0.3780	0.06	0.4897
	AV08	0.77	-0.33	0.0577	-0.05	0.8192
	AV09	0.77	0.25	0.2964	0.08	0.6650
	BP07	0.67	0.12	0.2267	-0.13	0.0614
	BP08	0.41	-0.22	0.3777	-0.17	0.4658
	BP09	0.78	**-0.53**	0.0059	-0.11	0.5503
	CM07	0.52	**-0.35**	0.0244	-0.13	0.0614
	CM09	0.68	0.00	0.9800	0.04	0.5331
	CS07	0.63	0.23	0.5264	-0.02	0.9611
	CS08	0.39	0.14	0.3444	-0.22	0.1461
	CS09	0.74	-0.11	0.5952	0.08	0.6583
	DW07	0.67	**-0.09**	0.0126	-0.05	0.0779
	DW08	0.60	-0.04	0.7398	0.11	0.1091
	DW09	0.79	**-0.31**	0.0128	0.10	0.3468
	HL07	0.54	-0.84	0.2472	-0.03	0.9486
	HL08	0.72	0.19	0.2129	0.09	0.1980
	HL09	0.74	0.18	0.2655	-0.13	0.2613
	IP07	0.75	-0.06	0.1448	0.02	0.7079
	IP09	0.78	0.06	0.8087	**0.65**	0.0094
	UP07	0.54	**-1.35**	<.0001	**-0.53**	0.0081
	UP09	0.65	0.26	0.4205	0.00	0.9913
Height	AV07	57.18	**0.30**	0.0026	0.05	0.6432
	AV08	53.86	**0.59**	0.0002	0.32	0.2700
	AV09	55.85	**0.59**	0.0093	0.19	0.5136
	BP06	92.18	**0.61**	0.0003	0.11	0.3769
	BP07	76.67	**0.40**	<.0001	0.12	0.1214
	BP08	57.92	**1.12**	<.0001	**0.94**	0.0003
	BP09	51.93	**1.03**	<.0001	**0.64**	0.0001
	CM06	68.18	**0.92**	<.0001	**0.79**	0.0056
	CM07	64.39	**0.77**	<.0001	0.12	0.1214
	CM09	77.83	**0.52**	0.0006	**0.54**	0.0002
	CS06	107.29	**0.36**	0.0004	**0.19**	0.0181
	CS07	85.53	**0.70**	0.0057	0.54	0.1102
	CS08	82.56	0.23	0.1018	0.18	0.2166
	CS09	69.55	**0.56**	0.0015	0.41	0.0610
	DW07	40.85	**0.14**	<.0001	0.06	0.1269
	DW08	57.82	**0.46**	<.0001	0.13	0.1095
	DW09	55.07	**0.78**	<.0001	**0.64**	<.0001
	HL06	116.24	**0.36**	0.0256	**0.46**	0.0024
	HL07	95.53	**2.24**	0.0008	0.90	0.3955
	HL08	54.91	**0.54**	<.0001	**0.33**	0.0196
	HL09	76.54	**0.68**	<.0001	0.23	0.2220
	IP07	37.03	**0.25**	<.0001	**0.22**	<.0001
	IP09	56.29	**0.44**	0.0382	0.02	0.9594
	UP07	103.05	**1.10**	0.0002	-0.28	0.1983
	UP09	101.83	0.38	0.1831	0.63	0.2108

Table 7 - continued

Trait	Population & Year	Trait Mean	Total selection (s)	P	Direct Selection (β)	P
LN Leaf #	AV07	2.79	**0.48**	<.0001	**0.47**	<.0001
	AV08	2.40	**0.51**	0.0020	0.32	0.0837
	AV09	2.63	**0.86**	<.0001	**0.76**	0.0012
	BP06	2.62	**1.05**	<.0001	**1.08**	<.0001
	BP07	2.35	**0.60**	<.0001	**0.56**	<.0001
	BP08	1.67	**0.88**	<.0001	**0.87**	0.0075
	BP09	2.53	**1.17**	<.0001	**0.88**	<.0001
	CM06	2.67	0.46	0.0790	0.26	0.2549
	CM07	2.52	**0.91**	<.0001	**0.56**	<.0001
	CM09	2.58	**0.86**	<.0001	**0.92**	<.0001
	CS06	2.37	**0.76**	<.0001	**0.78**	<.0001
	CS07	2.18	**1.37**	<.0001	**2.05**	<.0001
	CS08	1.94	**0.47**	0.0002	**0.87**	0.0004
	CS09	2.62	**0.48**	0.0093	**0.35**	0.0381
	DW07	2.37	**0.13**	0.0005	**0.10**	0.0003
	DW08	2.77	**0.65**	<.0001	**0.44**	<.0001
	DW09	2.77	**0.26**	0.0351	0.18	0.0673
	HL06	3.06	**0.45**	0.0034	**0.42**	0.0014
	HL07	3.27	**3.09**	<.0001	**3.26**	<.0001
	HL08	2.49	**0.64**	<.0001	**0.50**	<.0001
	HL09	2.75	**0.71**	<.0001	**0.53**	0.0056
	IP07	2.87	**0.12**	0.0045	**0.09**	0.0114
	IP09	2.84	-0.15	0.5270	-0.44	0.1066
	UP07	2.60	**1.94**	<.0001	**1.88**	<.0001
	UP09	2.07	**0.58**	0.0303	0.72	0.1293
SQRT Leaf Area	AV07	6.94	**0.21**	0.0374	0.00	0.9819
	AV08	5.19	**0.38**	0.0332	0.14	0.5167
	AV09	6.87	**0.47**	0.0450	0.16	0.5989
	BP06	8.47	**0.69**	<.0001	-0.10	0.4989
	BP07	7.02	**0.31**	0.0009	0.09	0.1941
	BP08	7.03	0.32	0.2022	-0.09	0.6449
	BP09	6.21	**0.81**	<.0001	0.23	0.2007
	CM06	4.58	-0.16	0.5428	-0.30	0.1949
	CM07	4.19	-0.16	0.3474	0.09	0.1941
	CM09	6.44	**0.64**	<.0001	-0.05	0.7229
	CS06	7.35	**0.35**	0.0012	0.02	0.7698
	CS07	5.22	**0.60**	0.0421	-0.47	0.2539
	CS08	4.94	0.28	0.0593	-0.01	0.9741
	CS09	7.12	**0.38**	0.0470	0.13	0.6031
	DW07	5.19	0.04	0.3515	0.04	0.2965
	DW08	6.45	**0.59**	<.0001	**0.30**	0.0026
	DW09	7.15	**0.67**	<.0001	**0.33**	0.0222
	HL06	7.34	0.06	0.7086	-0.23	0.0935
	HL07	7.12	**1.58**	0.0240	-1.07	0.3420
	HL08	5.41	**0.33**	0.0224	-0.14	0.3169
	HL09	8.33	**0.52**	0.0004	0.03	0.8708
	IP07	5.22	**0.12**	0.0036	0.07	0.1470
	IP09	5.30	0.27	0.2435	0.52	0.2077
	UP07	7.70	**0.94**	0.0017	-0.11	0.6098
	UP09	6.81	0.23	0.4329	-0.61	0.4722

Table 7 - continued

Trait	Population & Year	Trait Mean	Total selection (s)	P	Direct Selection (β)	P
LN Leaf Shape	AV07	0.91	0.06	0.5806	-0.03	0.7400
	AV08	1.04	0.18	0.3179	-0.03	0.9020
	AV09	0.88	0.01	0.9662	0.01	0.9544
	BP06	0.96	-0.39	0.1873	0.06	0.7082
	BP07	0.90	-0.05	0.6206	-0.03	0.6503
	BP08	0.93	0.27	0.2774	-0.27	0.1739
	BP09	1.03	-0.04	0.8182	-0.10	0.4107
	CM06	1.07	-0.11	0.6845	-0.15	0.5418
	CM07	1.00	-0.21	0.2101	-0.03	0.6503
	CM09	1.05	-0.23	0.1788	-0.15	0.1771
	CS06	0.87	-0.11	0.3248	-0.05	0.5209
	CS07	0.96	-0.27	0.3615	-0.17	0.6502
	CS08	1.07	-0.16	0.2709	-0.08	0.5498
	CS09	0.96	0.04	0.8399	0.14	0.4332
	DW07	0.88	**0.11**	0.0028	**0.07**	0.0270
	DW08	0.97	-0.06	0.6433	-0.04	0.5728
	DW09	0.97	-0.10	0.4496	0.04	0.7178
	HL06	0.75	0.24	0.1598	0.05	0.6746
	HL07	0.96	0.48	0.5144	0.17	0.8101
	HL08	0.93	0.10	0.5252	-0.01	0.8966
	HL09	0.93	0.05	0.7382	-0.07	0.6540
	IP07	0.94	-0.03	0.4654	-0.06	0.1243
	IP09	1.00	-0.15	0.5311	**-0.53**	0.0387
	UP07	1.02	-0.54	0.0778	-0.08	0.6150
	UP09	1.23	-0.02	0.9445	-0.54	0.4977
LN Leaf Thickness	AV07	-1.86	0.06	0.5586	-0.06	0.4147
	AV08	-1.44	**0.35**	0.0443	0.09	0.6184
	AV09	-1.82	0.27	0.2618	-0.12	0.5824
	BP07	-1.78	0.07	0.4849	0.02	0.7725
	BP08	-1.79	0.00	0.9954	-0.30	0.1962
	BP09	-1.89	**0.41**	0.0343	-0.17	0.2782
	CM07	-1.41	0.20	0.2349	0.02	0.7725
	CM09	-2.00	0.26	0.1276	-0.01	0.9153
	CS07	-1.86	0.35	0.2388	0.46	0.1316
	CS08	-1.63	0.12	0.4365	-0.02	0.8488
	CS09	-2.19	**0.51**	0.0053	0.08	0.7432
	DW07	-1.75	**-0.08**	0.0296	0.01	0.8149
	DW08	-1.51	0.03	0.7996	-0.03	0.7095
	DW09	-1.84	0.11	0.3775	-0.12	0.1807
	HL07	-1.90	1.27	0.0747	-0.13	0.8065
	HL08	-1.91	0.21	0.1705	0.13	0.0833
	HL09	-2.03	**0.44**	0.0041	0.10	0.3836
	IP07	-1.73	**0.09**	0.0264	-0.02	0.6502
	IP09	-1.96	0.24	0.3068	**0.66**	0.0469
	UP07	-1.71	-0.02	0.9513	-0.25	0.1195
	UP09	-2.11	0.29	0.3671	0.30	0.3514

Table 8: Total selection (selection differentials, s) and direct selection (selection gradients, β) from the *F. japonica* populations. Bold s and β values are significant at the $p \leq 0.05$ level. Trait means were calculated from the unstandardized data.

Trait	Population & Year	Trait Mean	Total selection (s)	P	Direct Selection (β)	P
ARCSIN % Germ	CM07	1.27	-0.11	0.5628	-0.18	0.4587
	LR07	1.27	-0.13	0.2272	-0.15	0.3862
	MP07	1.13	0.11	0.4561	0.09	0.5173
	NR07	1.32	-0.10	0.4836	-0.03	0.7676
	PG07	1.21	0.71	0.4677	0.17	0.9451
	SB07	1.13	-0.01	0.9622	0.05	0.7463
Avg Time to Germ	CM07	3.68	-0.01	0.9715	-0.04	0.9018
	LR07	3.69	0.15	0.1926	0.15	0.1481
	MP07	4.32	0.03	0.8270	0.05	0.8239
	NR07	3.77	-0.06	0.6469	0.11	0.2841
	PG07	3.99	-0.17	0.4983	0.46	0.4902
	SB07	3.60	-0.14	0.3247	-0.24	0.0710
Fv/Fm	CM07	0.48	0.00	0.9786	-0.03	0.9060
	CM08	0.79	-0.44	0.1981	-0.02	0.9633
	CM09	0.77	0.14	0.3103	-0.24	0.1931
	LR07	0.65	0.06	0.6096	-0.02	0.8731
	LR08	0.78	0.05	0.7962	0.02	0.7465
	LR09	0.77	**-0.35**	0.0332	-0.07	0.2993
	MP07	0.67	0.10	0.4901	0.04	0.8721
	MP08	0.77	0.04	0.7960	-0.19	0.0644
	MP09	0.80	0.02	0.8827	-0.05	0.4535
	NR07	0.58	-0.01	0.9702	-0.16	0.1388
	PG07	0.38	0.05	0.8179	-0.20	0.6946
	PG08	0.74	0.12	0.3899	0.02	0.8876
	PG09	0.68	-0.11	0.3441	-0.09	0.3742
	SB07	0.72	-0.04	0.7790	0.05	0.7467
	SB08	0.78	0.02	0.9245	-0.07	0.7444
	SB09	0.66	**-0.41**	0.0384	**-0.99**	<.0001
Height	CM07	276.63	0.18	0.3092	0.35	0.2567
	CM08	230.64	0.09	0.8152	0.09	0.7949
	CM09	275.03	0.21	0.1244	0.27	0.1531
	LR07	144.84	**0.26**	0.0140	0.24	0.0992
	LR08	173.09	**0.59**	<.0001	0.17	0.0872
	LR09	179.89	**0.52**	0.0006	0.05	0.5064
	MP07	218.21	**0.40**	0.0020	0.32	0.0743
	MP08	240.78	**0.39**	0.0012	0.04	0.7020
	MP09	248.31	**0.38**	0.0030	0.02	0.8754
	NR07	274.69	**0.27**	0.0327	-0.07	0.5960
	PG07	264.98	0.33	0.1304	0.62	0.2482
	PG08	222.98	**0.55**	<.0001	**0.45**	0.0006
	PG09	215.31	**0.46**	<.0001	**0.39**	0.0026
	SB07	263.18	0.23	0.0920	0.23	0.1208
	SB08	259.83	0.21	0.2711	0.22	0.2501
	SB09	280.33	0.31	0.1123	**0.37**	0.0084

Table 8 - continued

Trait	Population & Year	Trait Mean	Total selection (s)	P	Direct Selection (β)	P
Leaf Area	CM07	169.40	0.11	0.5586	0.08	0.7200
	CM08	140.14	**-0.76**	0.0040	-0.52	0.4351
	CM09	188.60	0.00	0.9738	0.18	0.3418
	LR07	124.01	-0.02	0.8508	-0.01	0.9257
	LR08	161.23	**0.41**	0.0127	**0.17**	0.0065
	LR09	188.85	0.21	0.2245	0.03	0.6854
	MP07	156.46	0.23	0.0984	-0.06	0.7620
	MP08	158.85	-0.05	0.6968	0.05	0.5461
	MP09	206.28	**0.34**	0.0104	0.10	0.2563
	NR07	193.19	0.20	0.1189	-0.10	0.4256
	PG07	166.35	-0.28	0.2082	-0.42	0.4906
	PG08	132.86	-0.16	0.2547	-0.02	0.8822
	PG09	158.45	0.11	0.3450	-0.06	0.5360
	SB07	141.81	-0.02	0.9080	0.00	0.9729
	SB08	169.88	-0.30	0.1032	-0.27	0.1062
	SB09	151.68	0.00	0.9830	0.27	0.0639
Leaf Shape	CM07	1.52	-0.13	0.4603	-0.18	0.6521
	CM08	1.58	**0.68**	0.0204	-0.12	0.8516
	CM09	1.57	**-0.31**	0.0165	**-0.32**	0.0495
	LR07	1.40	-0.19	0.0846	-0.15	0.2824
	LR08	1.39	0.09	0.6327	-0.08	0.2085
	LR09	1.57	0.03	0.8593	-0.04	0.5554
	MP07	1.65	-0.15	0.3012	-0.06	0.7329
	MP08	1.57	-0.13	0.3308	0.03	0.7881
	MP09	1.62	**-0.34**	0.0096	-0.06	0.4227
	NR07	1.51	-0.19	0.1515	-0.04	0.6796
	PG07	1.64	-0.24	0.2848	-0.49	0.2684
	PG08	1.55	-0.14	0.3176	-0.06	0.6200
	PG09	1.56	-0.14	0.2312	-0.06	0.5612
	SB07	1.43	-0.06	0.6923	-0.04	0.7901
	SB08	1.54	**-0.41**	0.0207	-0.30	0.1268
	SB09	1.62	0.09	0.6773	0.07	0.6158
Leaf Thickness	CM07	0.28	0.18	0.3248	0.22	0.5232
	CM08	0.29	0.49	0.1409	0.15	0.6074
	CM09	0.24	-0.13	0.3568	0.05	0.7126
	LR07	0.34	0.19	0.0826	0.05	0.5863
	LR08	0.34	-0.28	0.1007	-0.08	0.1466
	LR09	0.31	0.09	0.5959	-0.08	0.2406
	MP07	0.26	-0.20	0.1751	-0.06	0.7436
	MP08	0.28	0.01	0.9277	0.13	0.1185
	MP09	0.26	**0.33**	0.0142	0.10	0.2284
	NR07	0.27	0.03	0.8152	0.07	0.4566
	PG07	0.24	-0.21	0.3545	0.50	0.4564
	PG08	0.33	-0.03	0.8297	0.07	0.5514
	PG09	0.25	-1.16	0.6895	-0.15	0.9515
	SB07	0.28	0.00	0.9869	0.08	0.5542
	SB08	0.28	0.09	0.6402	0.01	0.9725
	SB09	0.25	0.24	0.2541	**0.43**	0.0035

Table 8 - continued

Trait	Population & Year	Trait Mean	Total selection (s)	P	Direct Selection (β)	P
SQRT Leaf #	CM07	6.95	-0.29	0.0980	-0.12	0.7821
	CM08	9.40	**0.80**	0.0011	0.39	0.5390
	CM09	9.07	0.20	0.1463	**0.35**	0.0362
	LR07	7.63	**0.30**	0.0024	0.05	0.7469
	LR08	8.45	**0.70**	<.0001	**0.50**	0.0001
	LR09	7.98	**0.69**	<.0001	**0.64**	<.0001
	MP07	6.53	**0.36**	0.0068	0.25	0.1459
	MP08	8.35	**0.52**	<.0001	**0.59**	<.0001
	MP09	8.80	**0.58**	<.0001	**0.44**	0.0007
	NR07	9.89	**0.43**	<.0001	**0.58**	0.0091
	PG07	7.56	0.29	0.1875	0.52	0.2341
	PG08	8.52	**0.48**	0.0002	**0.27**	0.0496
	PG09	8.55	**0.36**	0.0013	0.15	0.1963
	SB07	6.50	**0.34**	0.0062	**0.38**	0.0062
	SB08	9.39	**0.49**	0.0048	0.27	0.1827
	SB09	6.78	-0.08	0.7520	**0.67**	0.0050

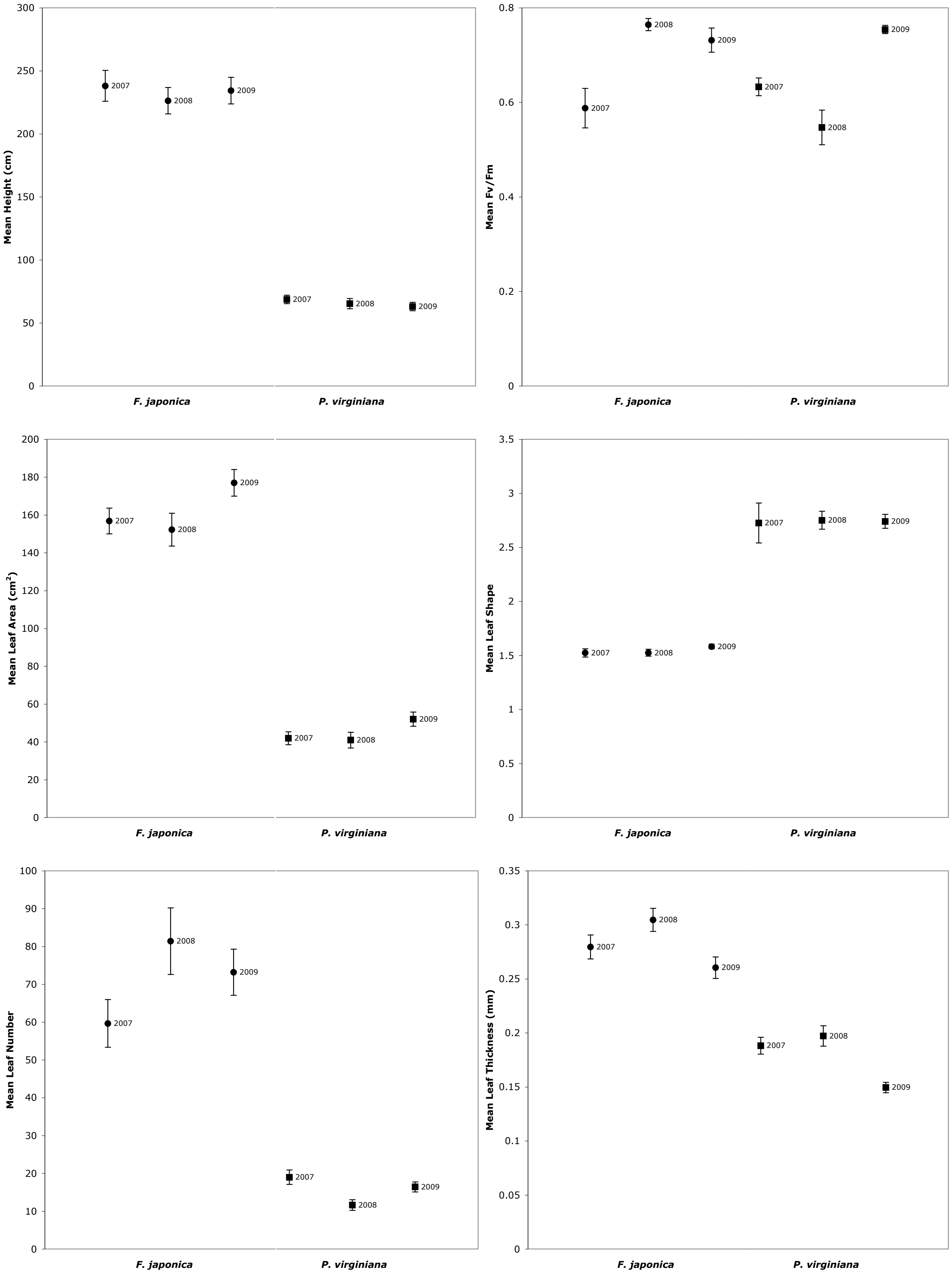

Figure 1: Comparisons of means of *Fallopia japonica* (circles) and *Persicaria virginiana* (squares) across three field seasons. Error bars are the 95% confidence intervals.

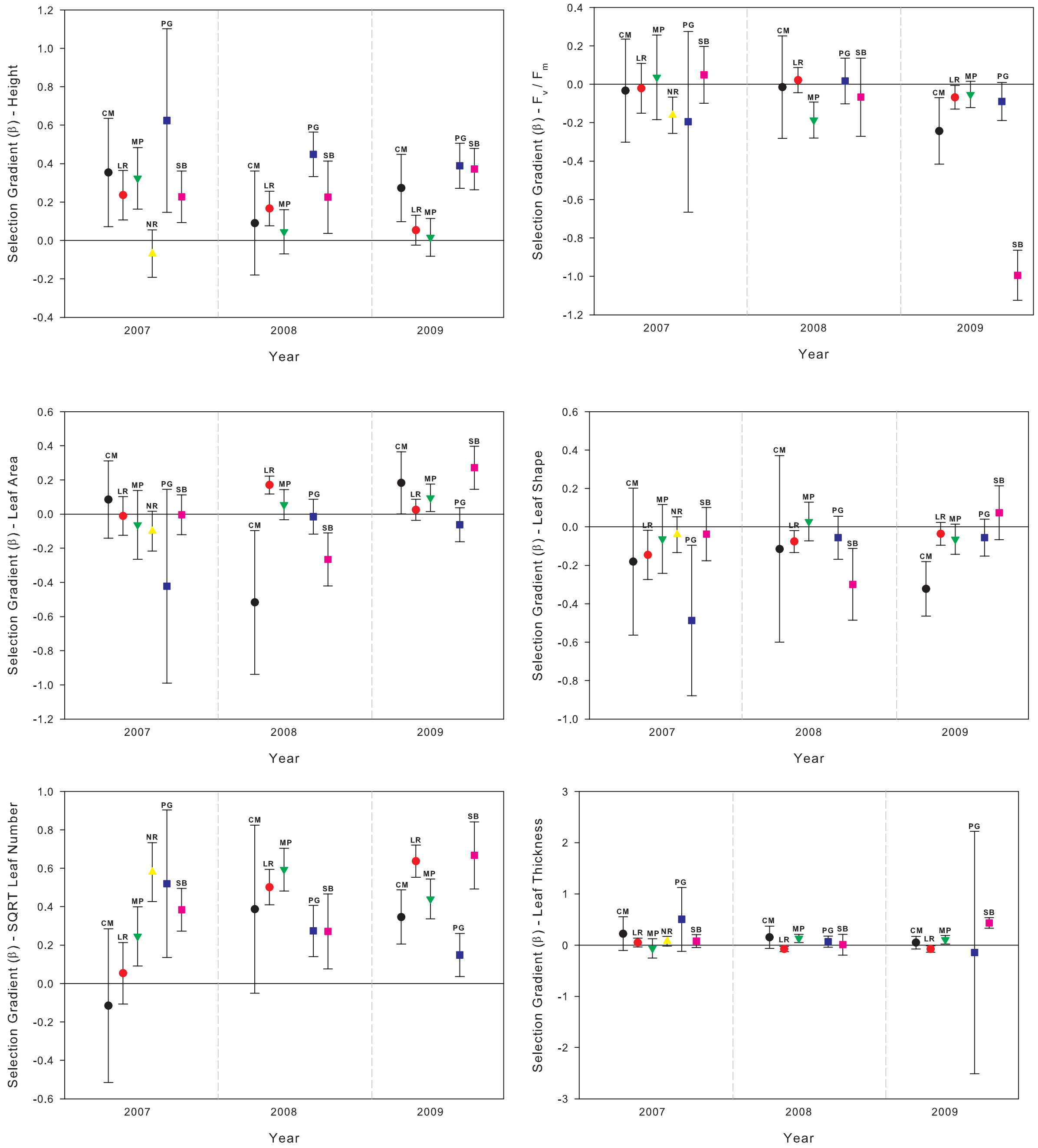

Figure 2: Selection gradients for *Fallopia japonica* populations for all three field seasons. Error bars represent ± one standard error. Populations are labeled with their two-letter designation (see Table 1). CM = black circle, LR = red circle, MP = green triangle, NR = yellow triangle, PG = blue square, SB = pink square.

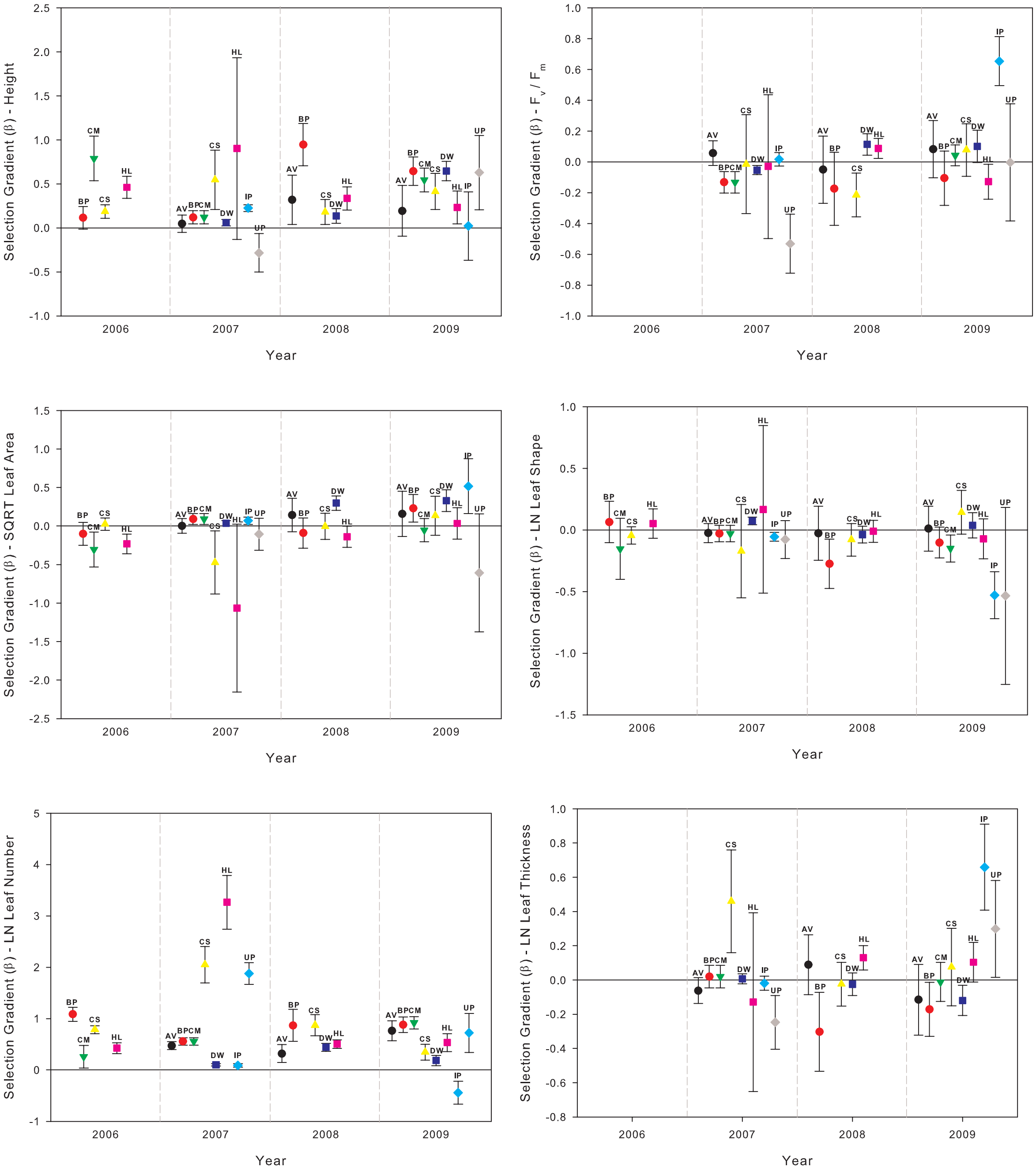

Figure 3: Selection gradients for *Persicaria virginiana* populations for all field seasons. Four populations (BP, CM, CS, HL) were measured as part of a pilot study in 2006. Error bars represent ± one standard error. Populations are labeled with their two-letter designation (see Table 1). AV = black circle, BP = red circle, CM = green triangle, CS = yellow triangle, DW = dark blue square, HL = pink square, IP = light blue diamond, UP = gray diamond.

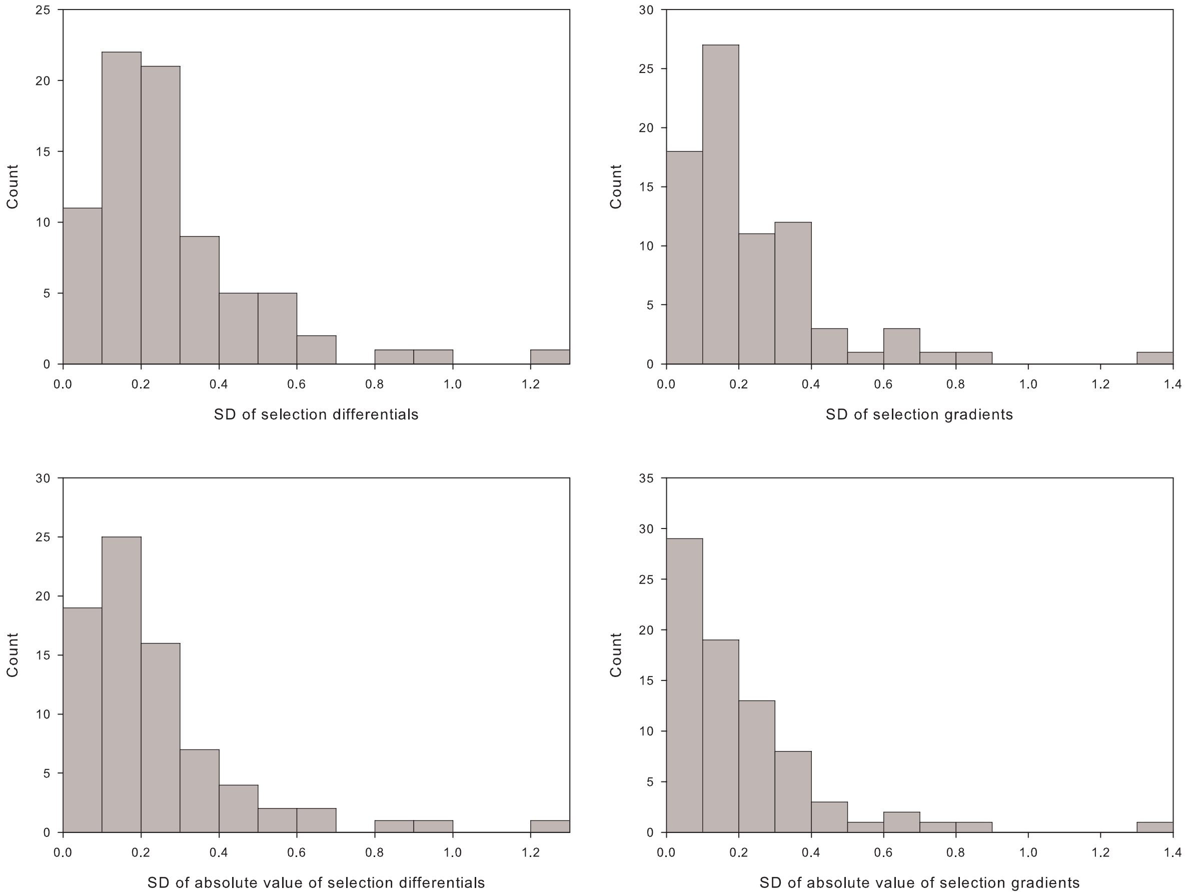

Figure 4: Histograms of the SD distributions for all selection coefficients in the present study. Figures on the left are differentials, figures on the right are gradients. The top two figures represent SD for total variation. The bottom two figures use the absolute values of selection coefficients and are therefore representative of variation in strength of selection. N = 78 for all.

Chapter 3

Does the invasive Japanese knotweed (*Fallopia japonica*) exhibit more local adaptation than its native relative woodland knotweed (*Persicaria virginiana*)? A reciprocal transplant study

Introduction

The study of local adaptation can yield insights into what is causing natural selection in wild populations and local adaptation studies have become a standard means to test adaptation hypotheses (Reznick and Ghalambor 2001). Local adaptation can be a mechanism of maintaining genetic variation in a species and has been implicated in sympatric speciation and species invasions (Kawecki and Ebert 2004). In fact, species introductions and subsequent invasions are quickly being recognized as a new system for studying rapid evolution (Keller and Taylor 2008). Sakai *et al.* (2001) suggest that one reason that some introduced species become invasive is that they possess the ability to rapidly adapt to new local conditions. If it is indeed the case, then analysis of invasions has important implications that can affect a variety of fields of study, such as conservation, climate change, and agriculture. For example, the creation of an agricultural habitat can unintentionally begin an evolutionary experiment by imposing strong and consistent selection through the strict timing of planting and harvesting, human-regulated resource availability, etc. Weinig (2005) conducted a study that compared agricultural and nonagricultural populations of the aggressive invader, *Abutilon theophrasti* (velvetleaf). She demonstrated that populations of *A. theophrasti* that had existed for less than 50 years were able to adapt to the planting schedule of the corn it was growing with (accelerated life history traits) and perform better in the cornfield than in the nonagricultural environment (Weinig 2005).

In order to illuminate the possible connection between local adaptation and introduced species, we must first show that there is the potential for evolution in populations of introduced species. Natural selection plays an obvious role, however the evolutionary perspective is often left out of studies of biological invaders (Keller and Taylor 2008). As an invasive species aggressively expands its range, it is likely to encounter environments that are different from its habitat of origin, setting the stage for adaptive evolution via natural selection. We have previously demonstrated that there is detectable natural selection occurring in natural populations of the invasive Japanese knotweed (*Fallopia japonica*) and its native relative, woodland knotweed (*Persicaria virginiana*) (O'Donnell 2010, Chapter 2). In a phenotypic selection analysis of multiple populations of each species, we found several traits that were consistently correlated with higher fitness: height, leaf number, and leaf size. Not only did we detect selection on these traits, but also that selection was strong (mean strength of selection gradients was 0.46 and 0.70 for *F. japonica* and *P. virginiana*, respectively).

Given that we know there is selection in the wild for both *F. japonica* and *P. virginiana*, we may ask whether these populations become locally adapted to their place of residence, and if so, which species displays greater local adaptation? To attempt to answer these questions, we used a classic reciprocal transplant experimental setup focusing on light availability as a potential selective agent given its importance in plant performance. There is a plethora of studies regarding plant adaptation and physiological response to different light regimes (Pearcy 1999), including local adaptation to photoperiod (Banta *et al.* 2007), response to neighbor shading (Tucnić *et al.* 2006), and many others. Reciprocal transplant experiments have been used for some time (see Clausen *et al.* 1940 for the classic example) and continue to help us untangle causal relationships with respect to selection in wild populations. Reciprocal transplants per se cannot show which specific traits are responsible for fitness differences experienced by transplanted plants. However, they can be matched with selection analyses (e.g., O'Donnell 2010, Chapter 2) to suggest traits that are likely responsible for local adaptation of the plants (Parker *et al.* 2002). A recent review (Herford 2009) suggests that local adaptation is common, although the magnitude of the fitness difference between home plants and transplanted plants may differ depending on what one is using as a fitness proxy.

We expect that, because in its native range, *F. japonica* is a primary colonizer of volcanic slopes and that it has been noted to grow best in full sun (Beerling *et al.* 1994), it will indeed respond to light as a selective agent. We hypothesize that the wide variety of light regimes that this species experiences in its invasive range represent novel habitat that can influence selection. If the ability to adapt locally is one factor that contributes to a successful invasion, as has been demonstrated by Weinig (2005), then we expect to find the *F. japonica* a least as locally adapted as its native relative which has been in this range for a longer period of time; that is, their fitness should be greater at home than at a transplanted site. If their fitness is the same regardless of the habitat that they are growing in, this suggests that the traits contributing to fitness are plastic and adjust from one site to another.

Phenotypic plasticity has also been implicated as a key to successful invasion (Maron *et al*. 2004). There is a general sense that invasions either involve a phenotypically plastic, general-purpose genotype or populations that adapt to local conditions rapidly (Parker *et al.* 2003). In a study of saltcedar invading colder habitat in North America, results showed support for an invasion model that includes both adaptive evolution and trait plasticity (Sexton *et al.* 2002). The results of this experiment will provide us with an insight into the relative importance of these two mechanisms and provide the basis for additional experiments comparing the relative levels of plasticity of these two species.

Methods

Study Species

Fallopia japonica (Houtt.) Ronse Decr is an erect, herbaceous perennial plant in the family Polygonaceae. It overwinters as an underground rhizome and buds for the next year's shoots form on the rhizome in the fall and remain underground for the winter (Beerling *et al.* 1994). Known by the common name, Japanese knotweed, this plant was recently placed in the genus *Fallopia* (Ronse Decraene *et al*. 2000), although much work on it continues to be published under its former designations, *Polygonum cuspidatum* (Siebold & Zucc.) Moldenke and *Reynoutria japonica* Houtt. *F. japonica* was introduced to England from eastern Asia in the early 19th century primarily as an ornamental plant (Connolly 1977), and from there it was brought to North America in the latter part of that century. On both continents, *F. japonica* quickly spread from the sites where it was introduced and is now considered highly invasive; it is currently a quarantined or prohibited species in nine states (USDA 2010) and has been listed as one of New York State's top invaders (IPC 2008). *F. japonica* grows dense stands of stems made all the more dense by the presence of previous years' growth, which takes a substantial time to break down. It primarily spreads vegetatively, although it does produce large amounts of viable seed (up to 90% germinate in a greenhouse setting (O'Donnell 2010, Chapter 2)). In a rhizome regeneration trial, *F. japonica* was able to grow from a piece of rhizome only 1 cm long and weighing only 0.7 g (Brock and Wade 1992). Even pieces of the stem have approximately a 30% regeneration rate when placed in moist conditions, putting out new shoots and adventitious roots within a month's time (Brock *et al.* 1995). In its native range in Eastern Asia, *F. japonica* is a pioneer of open volcanic slopes; in greenhouse trials, it performs best in full sun (Beerling *et al.* 1994). In its introduced range in New York, populations can be found under a variety of canopy types and in different light levels (see Table 2 in O'Donnell 2010, Chapter 2).

Persicaria virginiana (L.) Gaertn is also an erect herbaceous perennial that grows from an underground rhizome. It is about half the size of *F. japonica*, reaching heights of 1-2 m. *P. virginiana* is not listed on any noxious weed lists. *P. virginiana* is native to North America with a distribution from the midwestern United States to the east coast and up into eastern Canada. It is known by the common names woodland knotweed and jumpseed; the latter due to the way the achenes are projected from the inflorescence. When the stiff stigma are lightly touched, tension between two tissue layers in the pedicle cause the flower and achene to be projected up to 3 m from the inflorescence (Reed and Smoot 1906). As in *F. japonica*, winter buds form on the rhizome in the fall and overwinter underground before emerging in late spring. *P. virginiana* was also formerly included in the genus *Polygonum*, which has been divided on the basis of several lines of evidence, including floral morphology (Ronse Decraene and Akeroyd 1988) and DNA sequencing (Kim and Donoghue 2008). *P. virginiana* was chosen for comparison to *F. japonica* due to its similar growth habit (i.e. erect, rhizomatous perennial) and its relatedness. No other native *Fallopia* occur on Long Island in populations large enough for study that we could have used to make a congeneric comparison.

Locations and Planting

In the fall of 2008, *F. japonica* rhizomes were collected from Paul T. Given County Park (Smithtown, NY – 40°51’29.02” N, 73°12’41.36 W) and a yard waste field at the north end of Stony Brook University’s campus (Stony Brook, NY – 40°55’21.4” N, 73°7’23.59” W). *P. virginiana* rhizomes were collected from Caumsett State Park (Lloyd Neck, NY – 40°54’58.72” N, 73°28’1.99” W) and Bethpage State Park (Bethpage, NY – 40°45’5.76 N, 73°28’32.09” W). Sites were chosen out of those used in a previous study (O’Donnell 2010, Chapter 2) to maximize the difference in light regimes; a location summary is provided in Table 1. The Stony Brook *F. japonica* population grows under a thick canopy and is therefore exposed to darker conditions than the Smithtown population, which grows along the side of the Nissequogue River under fewer trees. In summer, these populations experience photosynthetically active radiation (PAR) levels of approximately 13.25 μmol s^{-1} m^{-2} and 228.26 μmol s^{-1} m^{-2}, respectively at 1 m height. This canopy situation parallels that of *P. virginiana*; the Bethpage population is in the interior of a wooded area under pines and oaks (73.76 μmol s^{-1} m^{-2}) and the Caumsett population is on the edge of an open field (96.8 μmol s^{-1} m^{-2}). All light measurements were taken between 11 am and 1 pm on days with less than 20% cloud cover using a LI-1000 DataLogger and Quantum Sensor (LI-COR, Inc., Lincoln, NE).

We harvested rhizome pieces from each population in October of 2008. For *F. japonica*, we collected 25 meter-long pieces of rhizome each from the Stony Brook site (SB) and the Smithtown site (PG). The individual rhizomes of *P. virginiana* are much smaller, so we collected rhizomes from 50 individuals each from the Bethpage site (BP) and the Caumsett site (CM). All rhizomes were brought back to the lab at Stony Brook University to be washed, cut, and weighed. When not being cleaned or measured, we stored the rhizomes in a 6°C refrigerator. *F. japonica* rhizomes were cut to approximately 8 cm lengths, as this size had the best regeneration percentage in the Brock and Wade (1992) trial. *P. virginiana* rhizomes were cut in half to create 100 pieces from each site. All rhizomes were tagged with aluminum markers and wires.

The PG and SB *F. japonica* rhizomes were reciprocally transplanted into plots at the site of the existing populations of *F. japonica*. Likewise, BP and CM *P. virginiana* rhizomes were reciprocally transplanted into the *P. virginiana* sites. Each site had two separate plots (2 m x 1 m) into which 50 rhizome pieces were planted randomly. Half of those were rhizomes that had originated at that site (replants) and half of them were from the other site for that species (transplants). The replants controlled for the effects of digging up, cutting, and replanting and allowed us to measure how well a plant should do in its habitat of origin. Once in the ground, the rhizomes were watered once and then left to natural conditions for one year's time. They were harvested in October 2009.

Measurements and Data Analysis

At the time of harvest, aboveground measurements were taken including: average height of stems and leaf thickness of the leaf at the tenth node from the base of each shoot. In the laboratory, rhizomes were washed and winter buds were counted. Total leaf area was measured using a LI-3100 Area Meter (LI-COR, Inc., Lincoln, NE). The leaf from the tenth node was used to estimate stomatal density. We painted a layer of clear nail polish on the abaxial side of the leaf, near the base of the blade. After drying, the polish was peeled off, mounted on a glass microscope slide, and viewed at 400X magnification with a compound light microscope (Alphaphot-2 Model YS2-T, Nikon Corporation, Japan). Stomata were counted at three different locations on the polish peel, and the average was taken and used to calculate the number of stomata per mm^2. All plant material was then dried for at least one week in a 160°F oven before final weighing. Dry rhizome and total aboveground mass were used to calculate a ratio of belowground to aboveground biomass, which will be hereafter called the root : shoot (R:S) ratio.

Before analysis, data were checked for normality and transformed if necessary (leaf area and total biomass were square root transformed and leaf thickness and root:shoot (R:S) were $\log_{10}$ transformed). We did an analysis of covariance with initial rhizome mass as the covariate using JMP (Version 7.0.1, SAS Institute Inc., North Carolina). The main effects were species (*F. japonica, P. virginiana*) and environment (shade, light), with population nested within species (PG and SB for *F. japonica*, BP and CM for *P. virginiana*) and plot (1, 2) nested within environment. We also included the following interaction terms: species by environment, plot by species, and population by environment.

We also performed a multiple regression selection analysis using final total biomass as the fitness proxy. Before the selection analysis, we checked traits for collinearity and removed some from the analysis as necessary. Developed by Lande and Arnold (1983), selection analyses of this type use regression to determine what traits are under selection and how strong the selection is. A linear regression of traits on fitness produces selection differentials (s) that quantify the total selection on that trait (i.e., selection directly on that trait plus any selection due to that trait's correlations with other traits). Multiple regression of all traits on fitness produces selection gradients (ß), which quantify direct selection on a trait while controlling for the effects of that trait's correlations with other traits (Lande and Arnold 1983). Relative fitness was calculated by dividing total biomass by the mean biomass for a plot and all other trait values were standardized by dividing them by the standard deviation of each trait in that particular plot. Initial rhizome mass was added to all regression models as a covariate. Note that we used the untransformed total biomass as the fitness proxy for the selection analysis, as it is improper to transform fitness values beyond making them relative (Lande and Arnold 1983). In instances of missing data preventing analysis for the multiple regressions, we changed the model from including all traits (height, LOG leaf thickness, SQRT leaf area LOG R:S, stomata density, and change in fresh rhizome mass) to including just height and LOG R:S.

Results

The results from the nested analysis of covariance show a clear (and not unexpected) effect of species on each trait, except for change in rhizome mass (Table 2). Means of absolute values of residuals from an ANCOVA of each trait with initial rhizome mass as a covariate are plotted in Figure 1; these adjusted means allow us to examine what the trait values would be if initial rhizome mass were held constant. Only for height was there an instance where a population performs better in its habitat of origin (Figure 1a), although the overlap of the standard errors is considerable and renders this result weak. In most cases, populations of the same species responded similarly, if they responded at all, to the different light habitats. Stomatal density, leaf thickness, total leaf area, and biomass are examples of this tendency (Figure 1b, c, d, e). Although the actual leaf area in the SB and PG *F. japonica* are different (untransformed means = 290 mm^2 and 177 mm^2 in the dark, respectively), both populations show a similar decrease in leaf area in the light environment. *P. virginiana* tended to have more stomata in the light, with a mean density of 39.31 stomata/mm^2 versus 24.61 stomata/mm^2 in the shade (Figure 1b). Biomass increased slightly in the light environment for both populations of *P. virginiana* (Figure 1e) and showed no difference across habitats for both populations of *F. japonica.* In these examples, both populations of each species responded in the same manner regardless of their origin, although they may have had different trait means in each habitat. For *P. virginiana*, R:S did not differ between populations or habitats (Figure 1f). The SB *F. japonica* does show an increase in R:S in the light environment, while the PG *F. japonica* shows only a minor increase.

Approximately twice as many selection differentials (23) as gradients (10) were significant, indicating more indirect selection on the measured traits (Table 3). The means of the absolute values of selection differentials and gradients were high at 0.30 and 0.25, respectively. In general, the majority of the significant differentials were for height, leaf area, and R:S ratio. Selection favored taller plants, with larger leaf areas, and greater allocation of resources to shoots (as shown by the negative selection coefficients). A single-factor ANOVA of the light and shade significant differentials and gradients showed no difference in the strength of selection between habitats. We examined the strengths of selection gradients by population in each site (Figure 2). There were a few cases in which the selection gradients were different for different populations of the same species in the same environment. In *P. virginiana*, for example, the gradients for height and R:S were different for each population at the shade environment and of similar strength in the light environment (Figure 2a, c). There seemed to be a tendency for the two populations of *P. virginiana* to have different gradients in the shade environment, but similar gradients in the light environment (Figure 2 all). For height, total leaf area, and stomatal density, most gradients were positive or zero regardless of environment (Figure 2a, d, e). Leaf thickness tended to have positive selection gradients in the shade environment only (Figure 2b).

Discussion

The issue of the role and importance of local adaptation to the invasion process is currently a much-discussed topic in evolutionary ecology (see Parker *et al.* 2003, Keller and Taylor 2008). The goal of this study was to find an answer to the question of how important local adaptation is to the invasive pest plant *F. japonica* compared to a native relative *P. virginiana.* We used a classic reciprocal transplant experiment focusing on light availability as a potential selective agent on traits related to light acquisition. If the ability to adapt locally is one factor that contributes to successful invasion, then we expected to find *F. japonica* to have a greater fitness at home than at a transplanted site. Additionally, if the invasive species is comparatively more locally adapted than its native relative *P. virginiana*, then the magnitude of the difference between transplants and replants should be greater for *F. japonica* than *P. virginiana.* If their fitness is the same regardless of the habitat that they are growing in, this suggests that the species may be generalists that can do well anywhere.

Our analysis showed very little, if any, local adaptation to light condition. In most cases, plants did not perform any better in their home site compared to the site into which they were transplanted, regardless of species. Indeed, if we look specifically at the effect of the different light regimes on biomass, which is used in the later analysis as our fitness proxy, we see both transplants and replants of *P. virginiana* had increased in biomass (almost to the same degree) in the light environment, with no response of biomass to the change in light regime in *F. japonica.* Both of these responses are suggestive of both species being habitat generalists for light regime. Height was the only trait for which the reaction norms for the populations of a species crossed each other, which would indicate that plants performed better at home and that there was potential genetically based differences between populations; however, the standard errors overlap too much to draw any strong conclusions about this relationship.

We did observe, however, that populations responded differently to the different light conditions, suggesting plasticity of those traits in response to light. In three of the populations, plants had greater total leaf area in the shade environment than in the light environment (BP *P. virginiana* is the exception having the same leaf area in light and dark). Physiologically, a plant will perform better in the shade by maximizing its photosynthetic area, and plasticity for total leaf area in response to shade has been shown in a variety of species such as *Oxalis acetosella* (Packham and Willis 1977), *Veronica* spp. (Dale and Causton 1992), and many others. In addition, having larger leaves in a higher light environment can mean more water loss via transpiration, which is detrimental during dry conditions.

In general, plants should have more stomata under light conditions than under shade conditions given their higher photosynthetic rates and stomatal conductance (Givnish 1988). In addition, stomata play an important role in thermoregulation of the leaves (Hart 1988). Our results for stomatal density in *P. virginiana* correspond with this physiological expectation; both CM and BP populations showed an increase in stomatal density in the light environment compared to the dark environment. However, the population that originated in the dark habitat (BP) had a greater stomatal density in both conditions compared to the population that originated in the light habitat (CM), which was surprising. It was also surprising that both the PG and SB populations of *F. japonica* showed a decrease in stomatal density in the light environment, which is opposite of what one would expect physiologically. Of course, this response could have been from an unmeasured environmental factor. Stomatal density can be affected not only by light intensity, but also by climatic attributes of a habitat (e.g., humidity, precipitation, etc.) and CO_2 concentration in the air (Casson and Gray 2008). More controlled experiments (i.e. in a growth chamber) would be needed to examine the differences in stomatal density further.

Biomass differences are often used as the overall indicator of whether or not a plant is locally adapted to its habitat. Our results did not show an effect of origin on biomass; both *P. virginiana* populations did better in the light and the *F. japonica* populations had approximately the same biomass in shade and light conditions. Local adaptation can be limited by genetic drift and gene flow (Hereford 2009), and these may contribute to the results we reported, although gene flow may not be as influential a factor given *F. japonica*'s propensity for vegetative reproduction. These species may also be habitat generalists or possess so-called general-purpose genotypes. In a reciprocal transplant of the invasive weed *Verbascum thapus*, results showed very little support for rapid adaptation in physiological traits to different elevations and much support for plasticity in the traits measured (Parker *et al.* 2003).

We also used biomass as the fitness proxy in our phenotypic selection analysis. One reason to pair selection analyses and reciprocal transplants is to get a better idea of what the specific selecting agent may be (Mitchell-Olds and Shaw 1990, Pigliucci and Kaplan 2006). As is often the case, most of the selection on our traits of interest was indirect, which is the result of a trait's correlation with other characters (both measured and unmeasured). If we measured these other characters, then the correlation was compensated for in the calculation of the selection gradients via multiple regression. However, as in all selection analyses, there is the problem of unmeasured traits that may affect the selection gradients without our knowledge (Mitchell-Olds and Shaw 1987).

Despite the fact that certain trait conditions are favored physiologically over others in shade versus light conditions, often our selection gradients did not match these expectations. For example, there was significant selection for an increase in leaf area even in populations that were growing in the light habitat, where it is advantageous to have comparatively small leaves. Similarly with the selection gradients for R:S ratio, where we expect a higher ratio in the light (Daubenmire 1974), selection gradients are negative in the light environment. We must extrapolate from the results of this selection analysis with care because it only contained data from a single growing season. Selection gradients are highly variable at these locations (O'Donnell 2010, Chapter 2), and there could be chance variation of some factor that influenced the correlations we found between traits and biomass (fitness).

The implications of our results for integrating an evolutionary perspective into invasive plant biology are that it may not merely be the distinction between a general purpose genotype and a habitat specialist that makes one species more invasive over another and that selection gradients may not help species become locally adapted even if the gradients are generally strong. Further experimentation under more controlled conditions (e.g., a common garden experiments) will allow us to examine more closely how different light regimes cause different patterns of plastic responses and selection pressures.

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Table 1: Field sites and illumination. The second measurement at the Caumsett site was made parallel to the ground, facing the field.

Species	*Location*	*Latitude*	*Longitude*	*Light*	*Habitat*
F. japonica	Paul Given County Park, Smithtown, NY	40°51’29.02”N	73°12’41.36”W	228.26	Under full canopy
F. japonica	Stony Brook University Campus, Stony Brook, NY	40°55’21.14”N	73°7’23.59”W	13.25	Open river edge
P. virginiana	Bethpage State Park, Bethpage, NY	40°45’5.76”N	73°28’1.99”W	73.76	Under full canopy
P. virginiana	Caumsett State Park, Lloyd Neck, NY	40°54’58.72”N	73°28’32.09”W	96.8 (111.14)	Open field edge

Table 2: Results of analysis of covariance of traits with initial rhizome biomass as the covariate. * P≤0.05, **P≤0.01, ***P≤0.001

	Trait						
Effects	Height	Stomatal density	LOG leaf thick.	SQRT leaf area	SQRT biomass	LOG R:S	Change in rhizome weight
SPECIES							
df	1	1	1	1	1	1	1
MS	100684.2	15665.0831	0.09880041	104.894312	21.8499721	27.1223636	44.8343923
F	**192.7895*** **	**34.2277282*** **	**6.56144007***	**3.89844479***	**63.4412721*** **	**184.082387*** **	3.4870718
ENVIRON							
df	1	1	1	1	1	1	1
MS	3136.5	9355.99091	0.07205093	28.9916106	1.26672003	0.83327231	219.664709
F	**6.0058***	**20.4425545*** **	**4.78497878***	1.07748639	3.67791454	**5.65550844***	**17.0847998*** **
POP[SPECIES]							
df	2	2	2	2	2	2	2
MS	582.6	1527.7415	0.01881311	123.720743	4.25894601	0.08615154	58.5536697
F	1.1155	**3.33806853***	1.24939893	**4.59813765***	**12.365826*** **	0.58471975	**4.55411215***
PLOT[ENVIRON]							
df	1	2	2	2	2	2	2
MS	2497.5	849.875106	0.05624833	163.880535	1.42208884	0.10641257	183.824217
F	**4.7822*** **	1.85695115	**3.73551144***	**6.0906946****	**4.12902703***	0.72223352	**14.2972439*** **
SPECIES*ENVIRON							
df	1	1	1	1	1	1	1
MS	1068.8	5296.23334	0.00037638	101.290748	2.63178519	0.01448527	220.261793
F	2.0466	**11.5721081*** **	0.02499594	**3.76451669***	**7.64137363****	0.09831308	**17.1312389*** **
PLOT*SPECIES[ENVIRON]							
df	2	2	2	2	2	2	2
MS	1273.3	111.360604	0.03612107	218.522781	1.73140828	0.88419786	76.6321151
F	2.4381	0.24331952	2.39883861	**8.12149852*** **	**5.02713428****	**6.00114559****	**5.96019427****
POP*ENVIRON[SPECIES]							
df	2	2	2	2	2	2	2
MS	349.1	936.948663	0.0064107	111.861819	0.10651865	0.11395795	11.9736927
F	0.6685	2.04720422	0.4257417	**4.15739537***	0.30927631	0.77344481	0.9312745
INITIAL rhizome biomass							
df	1	1	1	1	1	1	1
MS	15798	3399.64134	0.02049915	712.681409	192.30425	0.05536212	891.182796
F	**30.2499*** **	**7.42811251****	1.36137058	**26.4871286*** **	**558.354317*** **	0.37574864	**69.3132714*** **

Table 3: Selection coefficients from phenotypic selection analysis. Means were calculated from untransformed, unstandardized data. Bold indicates significance, *P≤0.05, **P≤0.01, ***P≤0.001. [a] designates gradients from adjusted model, see text. [b] indicates significance unable to be calculated. NE indicates not estimable coefficients.

		Pop/Env	*Mean* *(untransformed)*	*Selection Differential*		*Selection Gradient*	
Height	*P. virginiana*	BP/shade	68.92	**0.25**	**	**0.24**	**
		BP/light	57.91	**0.60**	***	0.26	
		CM/shade	73.94	**0.13**	**	0.00	
		CM/light	63.67	**0.26**	***	**0.22**	*
	F. japonica	PG/shade	33.98	**0.33**	**	0.10	
		PG/light	26.32	**0.19**	*	-0.02	
		SB/shade	25.87	**0.34**	***	0.10[a]	
		SB/light	26.68	0.31		0.16[a]	
LOG Leaf thickness	*P. virginiana*	BP/shade	0.19	0.00		0.05	
		BP/light	0.15	0.22		-0.03	
		CM/shade	0.18	-0.02		0.00	
		CM/light	0.15	0.01		0.00	
	F. japonica	PG/shade	0.21	**0.28**	***	**0.21**	***
		PG/light	0.21	0.00		-0.10	
		SB/shade	0.22	-0.06		0.06[b]	
		SB/light	0.16	NE		NE	
LOG Root:Shoot	*P. virginiana*	BP/shade	3.25	**-0.14**	*	**0.27**	*
		BP/light	4.39	**-0.59**	***	-0.25	
		CM/shade	2.40	**-0.20**	***	**-0.22**	***
		CM/light	5.30	**-0.35**	***	-0.04	
	F. japonica	PG/shade	24.12	**-0.36**	***	-0.03	
		PG/light	30.67	-0.05		-0.07	
		SB/shade	18.65	**-0.29**	***	-0.23[a]	
		SB/light	27.26	**-0.06**	*	**-0.05**[a]	*
SQRT Total leaf area	*P. virginiana*	BP/shade	143.46	**0.34**	***	**0.42**	***
		BP/light	110.49	**0.54**	***	0.27	
		CM/shade	297.77	**0.12**	*	0.07	
		CM/light	135.66	**0.31**	***	**0.29**	***
	F. japonica	PG/shade	290.02	**0.60**	***	**0.32**	*
		PG/light	123.63	**0.16**	*	**0.31**	*
		SB/shade	177.06	**0.33**	**	1.00[b]	
		SB/light	4.00	NE		NE	
Stomatal Density	*P. virginiana*	BP/shade	68.71	0.02		0.02	
		BP/light	116.52	**0.15**	**	0.03	
		CM/shade	67.22	0.02		0.00	
		CM/light	100.70	0.05		0.02	
	F. japonica	PG/shade	59.00	0.06		0.01	
		PG/light	65.58	-0.03		0.00	
		SB/shade	53.89	0.03		-0.09[b]	
		SB/light	62.50	NE		NE	

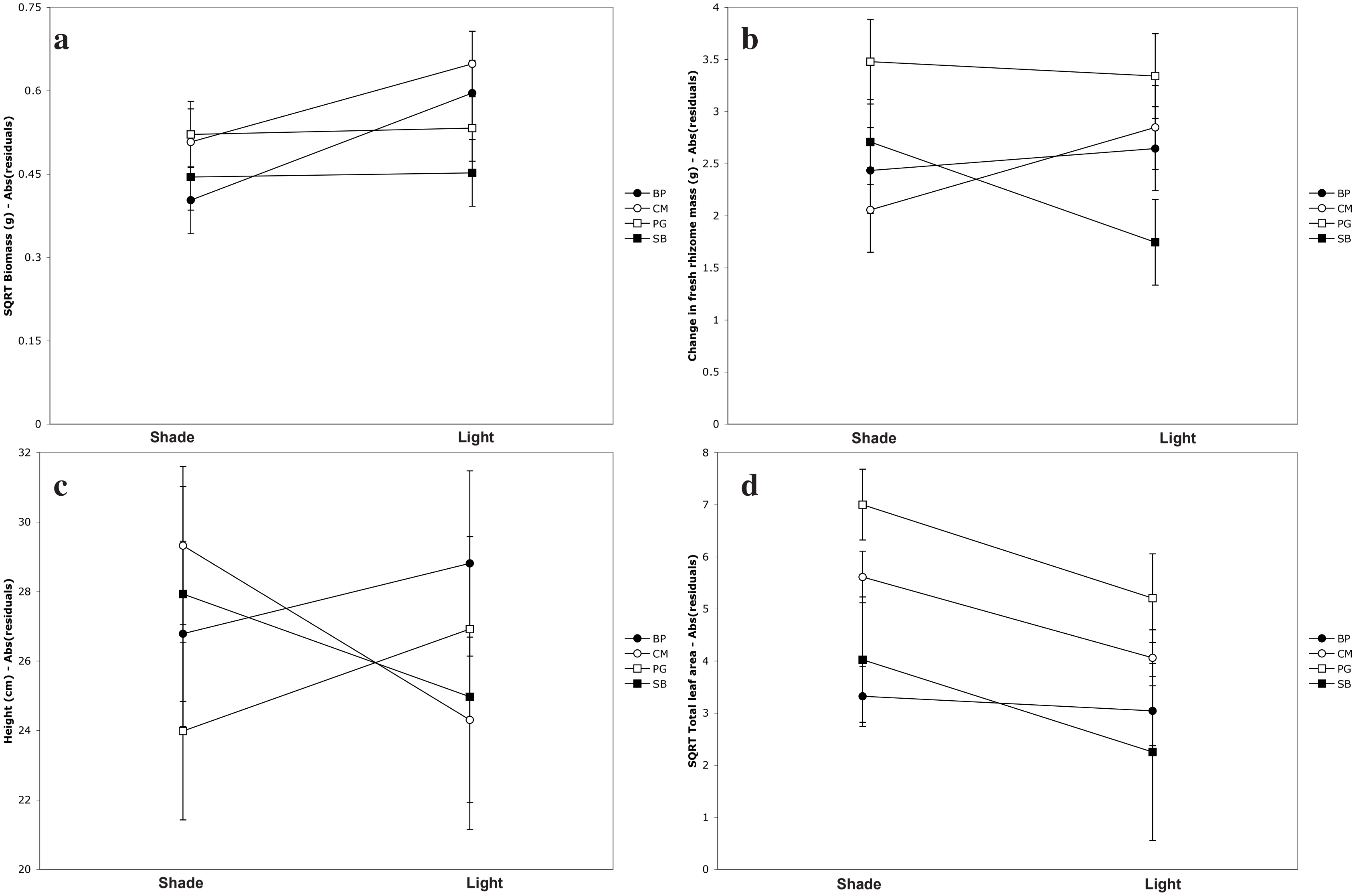

Figure 1 (a-d): Adjusted means from the residuals of an analysis of covariance with initial weight as the covariate. Bars are ± 1 SE. Squares are *F. japonica*, circles are *P. virginiana*. Closed and open symbols indicate origination from shade and light sites, respectively.

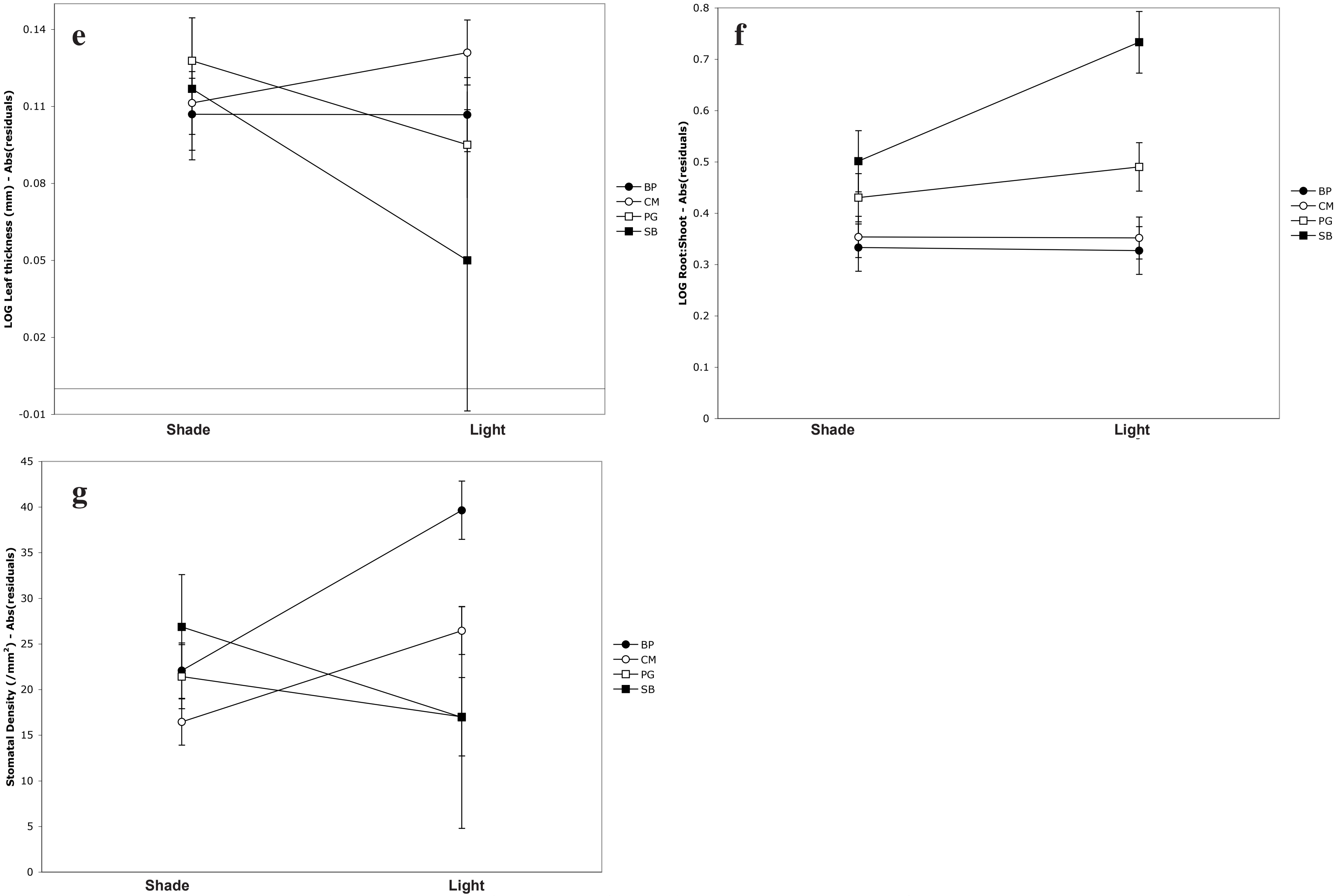

Figure 1 (e-g): Adjusted means from the residuals of an analysis of covariance with initial weight as the covariate. Bars are ± 1 SE. Squares are *F. japonica*, circles are *P. virginiana*. Closed and open symbols indicate origination from shade and light sites, respectively.

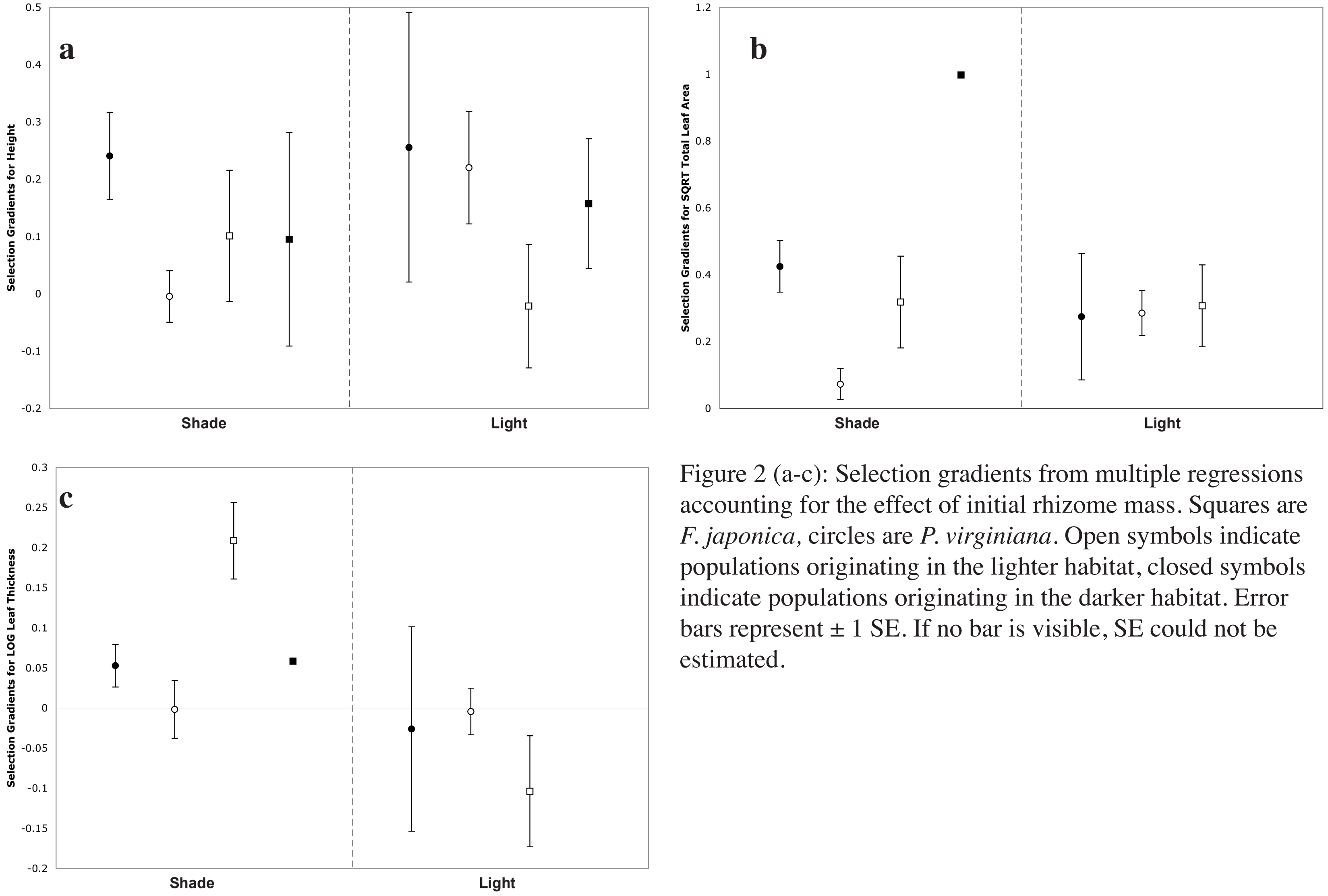

Figure 2 (a-c): Selection gradients from multiple regressions accounting for the effect of initial rhizome mass. Squares are *F. japonica,* circles are *P. virginiana*. Open symbols indicate populations originating in the lighter habitat, closed symbols indicate populations originating in the darker habitat. Error bars represent ± 1 SE. If no bar is visible, SE could not be estimated.

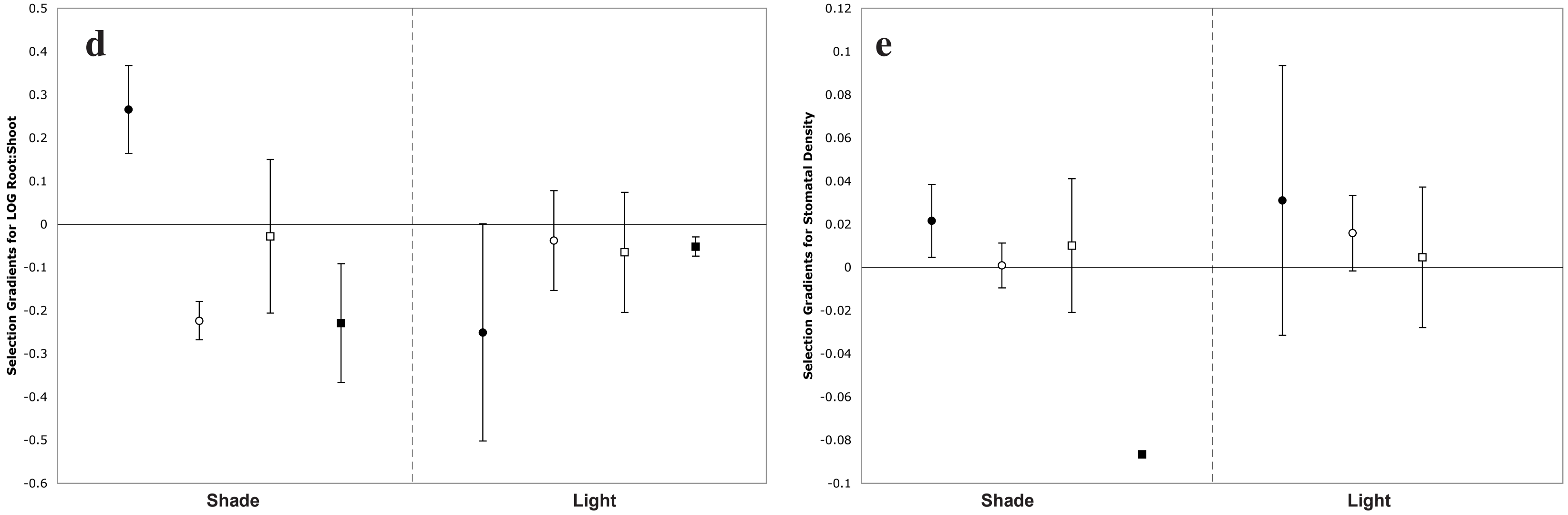

Figure 2 (d-e): Selection gradients from multiple regressions accounting for the effect of initial rhizome mass. Squares are *F. japonica,* circles are *P. virginiana*. Open symbols indicate populations originating in the lighter habitat, closed symbols indicate populations originating in the darker habitat. Error bars represent ± 1 SE. If no bar is visible, SE could not be estimated.

Chapter 4

Phenotypic plasticity in relation to light acquisition in the invasive weed *Fallopia japonica* (Japanese knotweed) and a noninvasive relative, *Persicaria virginiana* (woodland knotweed)

Introduction

A central problem in invasion biology is to identify the factor or factors that allow species to become invasive. While it is not likely to be a single factor, it is important to determine whether there are general traits associated with invasiveness. Baker (1965) famously proposed that a key innovation for an ideal weed would be to possess a so called "general purpose genotype" that would be successful in a variety of different habitat types. This lead to several studies on how phenotypic plasticity affects invasion by exotic species. However, these are often found lacking in critical aspects such as a clear definition of what the authors mean by plasticity and/or proper discussion of what it means when plasticity is not found in some traits (Richards *et al.* 2006). To be clear with our terminology, phenotypic plasticity refers to the potential of a trait (and not a whole organism) to respond to different environmental conditions (Pigliucci 2001). If the plasticity of a trait has a correlated effect on the fitness of an invasive organism, then it is capable of having an effect on that organism's invasion process (Richards *et al.* 2006), and it is within this context that the following work is done.

There are studies that propose that invasive plants may have wider ecological breadth due to plastic responses that allow them to be more successful in more environments (see Sultan *et al.* 1998, Sultan 2001, Saldaña *et al.* 2007 for examples). However, conflicting results have been found (Greer and McCarthy 1999, Gonzáles and Gianoli 2004), and so the best we can do at the moment is propose this as simply one of the possible mechanisms by which plasticity influences invasions. Of course, it most likely is the case that there are multiple (and not mutually exclusive) ways in which plasticity and invasion interact. Another proposal is that phenotypic plasticity is important primarily at the beginning of an invasion, when a species first encounters potentially novel environmental attribute. Simpson (1953) dubbed this the Baldwin effect, after J. Mark Baldwin proposed that plastic responses might be of primary importance in the establishment of a species, although he was primarily concerned with humans (Baldwin 1896). A good, empirically tested example of this is Yeh and Price's (2004) work on a population of dark-eyed juncos that became established in a mild, coastal environment from a montane habitat with a much shorter breeding season. They documented plastic changes in the birds' breeding season that allowed them to maintain a constant population size, thus aiding in their establishment (Yeh and Price 2004).

We used the framework set forth by Richards *et al.* (2006) with respect to the kinds of plastic responses that might be beneficial to an invasive species. A Jack-of-all-trades can maintain a somewhat constant level of fitness in a variety of environments due to plasticity of traits. A Master-of-some species possesses plasticity in traits that allow it to have higher fitness in good quality environments. There are also cases in which a species could be a Jack-and-master, possessing qualities of both categories (Richards *et al.* 2006). An organizing framework such as this one allows us to place our work in the context of what has already been done, thereby allowing us to see if there are any generalizations that can be made about invasions and phenotypic plasticity.

In this study, we make a comparison between the notorious invasive Japanese knotweed (*Fallopia japonica*) and a related native species, woodland knotweed (*Persicaria virginiana*) with respect to traits specifically related to light acquisition. We chose light because of its central role in photosynthesis and productivity, and therefore, fitness. Plasticity to light regime has been studied in detail for a group of annual weeds in the genus *Polygonum*, and it has been demonstrated that they may have wider ecological breadth due to plastic responses that allow a plant to be more successful in more environments (Sultan 2001). Different light regimes have well-known physiological and morphological effects on plants, including changing stomatal density, leaf thickness, nutrient allocation, and many others (Givnish 1988). By focusing on traits that are affected by differing light intensities and that are themselves affecting fitness, we can draw stronger conclusions about how plasticity of certain traits could influence the invasiveness of Japanese knotweed or lack thereof in woodland knotweed.

We set up a common garden experiment in the greenhouse to test for the effects of differing light regimes on several morphological and physiological traits in these two plants. Individuals from four populations of each species, *F. japonica* and *P. virginiana*, were planted in two light treatments to compare population level and species level plasticity for these traits. We asked the following questions: (i) Can we detect trait plasticity in response to light in these two species? (ii) How is the relative fitness of these species related to the plasticity we find? (iii) Based on those results, can we place the invasive *F. japonica* into the category of Jack-of-all-trades or Master-of-some?

Methods

Study Species

Fallopia japonica (Houtt.) Ronse Decr (common names: Japanese knotweed, American bamboo) is an herbaceous perennial in the family Polygonaceae that overwinters as a rhizome underground. Winter buds form on the rhizome in the fall and remain underground for the winter, emerging as new shoots in the spring (Beerling *et al.* 1994). The taxonomy of *F. japonica* has a complex history. It has been most recently placed in the genus *Fallopia* (Ronse Decraene *et al*. 2000), although work on it continues to be published under its former designations, *Polygonum cuspidatum* (Siebold & Zucc.) Moldenke and *Reynoutria japonica* Houtt. *F. japonica* grows in dense stands of erect stems that create a substantial canopy. It primarily spreads vegetatively, although it does produce large amounts of viable seed (up to 90% germinate in a greenhouse setting (O'Donnell 2010, Chapter 2)). *F. japonica* can grow from a piece of rhizome only 1 cm long and weighing only 0.7 g (Brock and Wade 1992). Even pieces of the stem have approximately a 30% regeneration rate when placed in moist conditions, putting out new shoots and adventitious roots within a month of planting (Brock *et al.* 1995).

Thought to have a pleasing shape and delicate flowers, *F. japonica* was brought to England from Japan by Phillipe von Siebold in the early 19th century as an ornamental plant (Conolly 1977) and was even awarded a medal for being the most interesting new ornamental plant of the year by the Society for Agriculture and Horticulture at Utrecht (Bailey and Conolly 2000). After heavy promotion by influential gardeners and several escapes from gardens, *F. japonica* began to spread throughout the British Isles (Conolly 1977) and was intentionally introduced multiple times into North America (Barney 2006). On both continents, *F. japonica* quickly spread, mostly clonally, from sites where it was planted and is now considered a highly invasive noxious weed; it is currently a quarantined or prohibited species in nine states (USDA 2010) and has been listed as one of New York State's invaders of primary concern (IPC 2008).

Persicaria virginiana (L.) Gaertn is an erect herbaceous perennial that grows from an underground rhizome similar to *F. japonica*, however, it is much smaller, native to North America and not considered a invasive pest species. *P. virginiana* has a distribution from the midwestern United States to the east coast and up into eastern Canada (Hinds and Freeman 2005). It is known by the common names woodland knotweed and jumpseed; the latter because achenes can be projected up to three meters from the inflorescence by a tension build up between two tissue layers in the pedicle (Reed and Smoot 1906). As in *F. japonica*, winter buds form on the rhizome in the fall and overwinter underground before emerging in the late spring. *P. virginiana* was also formerly included in the genus *Polygonum* and has also experienced taxonomic shuffling, and is now placed in the genus *Persicaria*, which has been divided up based on several lines of evidence, including floral morphology (Ronse Decraene and Akeroyd 1988) and DNA sequence data (Kim and Donoghue 2008). *P. virginiana* was chosen for comparison to *F. japonica* due to its similar growth habit (erect, rhizomatous perennial) and its relatedness. No other native *Fallopia* occur on Long Island in large enough populations to make a congeneric comparison that would minimize the effect of differing evolutionary histories (Muth and Pigliucci 2006).

Locations and Greenhouse Planting

In October 2009, *F. japonica* and *P. virginiana* rhizomes were collected from eight locations (four per species, see Table 1) from populations that had previously been used in a phenotypic selection analysis (O'Donnell 2010, Chapter 2): Caumsett State Park (CM), Setauket Mill Pond (MP), Paul T. Given County Park (PG), and a yard waste dump at Stony Brook University (SB) for *F. japonica*; and Bethpage State Park (BP), Caumsett State Park (CM), Caleb Smith State Park (CS), and The Nature Conservancy's

Uplands Farm Sanctuary (UP) for *P. virginiana.* Sites were variable for canopy cover and light levels (Table 1), ranging from full canopies and low photosynthetically active radiation (PAR) (i.e., 27.36 μmol s^{-1} m^{-2} and 27.43 μmol s^{-1} m^{-2} for *F. japonica* and *P. virginiana* populations, respectively) to open, sunny areas with high PAR (612.20 μmol s^{-1} m^{-2} and 721.63 μmol s^{-1} m^{-2} for *F. japonica* and *P. virginiana* populations, respectively). All PAR measurements were taken between 11 am and 1 pm on days with less than 20% cloud cover using a LI-1000 DataLogger and Quantum Sensor (LI-COR, Inc., Lincoln, NE).

We collected approximately 50 meter-long pieces of rhizome from each *F. japonica* population. Rhizomes of *P. virginiana* are much smaller, so we collected approximately 100 rhizomes from each population. All rhizomes were brought back to the lab at Stony Brook University to be washed, cut, and weighed. When not being cleaned or measured, we stored the rhizomes in a 6°C refrigerator. *F. japonica* rhizomes were cut to approximately 8 cm lengths, as this size had the best regeneration percentage in the Brock and Wade (1992) trial. *P. virginiana* rhizomes were cut to similar size. All rhizomes were tagged with aluminum markers.

To test the effect of light on responses of traits in both species, we set up a common garden experiment with two light levels in the greenhouse at Stony Brook University. Half of the greenhouse bay was covered with 50% shade cloth and half was left open to ambient light conditions (average PAR at mid-day: 374.8 μmol s^{-1} m^{-2} and 922.3 μmol s^{-1} m^{-2}). We planted in December 2009 and ran the experiment through the winter and spring, keeping the bay at approximately 27°C to mimic summer temperature conditions on Long Island. Within each treatment, we had three randomized blocks for each species. Rhizomes from the four populations of each species were randomized and planted in 6 inch round pots in Sunshine Mix 1 medium (Sun Gro Horticultural Canada, Ltd.) and watered approximately three times a week for the duration of the experiment. At three months, all plants were fertilized with Jack's LX Water-Soluble Fertilizer 15-5-15 Ca-Mg (Product #77940, J.R. Peters, Inc., Allentown, PA) delivered at a concentration of 1:100. Plants were checked every other day for shoot emergence and measurements (see below) were taken once per month. Both species were allowed to grow for five months after their shoots started emerging (five months after initial planting for *F.*

japonica, six for *P. virginiana*). At harvest, stems and leaves were collected and rhizomes and roots were washed. All material was dried in paper bags in a 70°C drying oven for at least five days for *F. japonica* and three days for *P. virginiana.*

Measurements and Data Analysis

During the course of the experiment, we counted leaves and measured height once a month. Before harvesting and drying aboveground material, we took a representative leaf (first above the 10th node) and measured F_v/F_m, stomatal density, and leaf area. F_v/F_m is a physiological measurement of efficiency proportional to the maximum quantum yield of the photosynthetic apparatus (Krause and Weis 1991). F_v is the difference between the constant or initial fluorescence (the state after a dark period when all photosynthetic reaction centers are open) and the maximum fluorescence in light (Krause and Weis 1984). F_v/F_m was measured with a chlorophyll fluorometer (Model OS-30p, Opti-Sciences, Inc., Hudson, NH). Leaf area was calculated by creating .jpg files of scanned leaves, importing them into ImageJ software (NIH, Bethesda, MD), and having it determine leaf area based on a calibrated centimeter scale. To estimate stomatal density, we placed a layer of clear nail polish on the abaxial side of the leaf, near the base of the blade and allowed it to dry. The polish was peeled off, mounted on a glass microscope slide, and viewed at 400X magnification with a compound light microscope (Alphaphot-2 Model YS2-T, Nikon Corporation, Japan). Stomata were counted at three different locations on the polish peel and the average was taken and used to calculate the number of stomata per mm^2. The traits chosen are likely to be important to a plant's fitness as they all are related to light acquisition and photosynthesis. Dry rhizome and aboveground mass were used to calculate the ratio of belowground to aboveground biomass, which hereafter will be referred to as the root : shoot (R:S) ratio. Growth rate was determined by using Microsoft Excel (Microsoft Office 2004 for Mac, Microsoft Corp., Redmond, WA) to calculate the slope of the line that best fit the distribution of height and age (measured as the days since the first shoot emerged) of each individual plant.

Before analysis, data were checked for normality and transformed if necessary (total biomass, leaf number, and growth rate were square root transformed and stomatal density was $\log_{10}$ transformed). We did an analysis of covariance with initial rhizome mass as the covariate using JMP (Version 7.0.1, SAS Institute Inc., North Carolina). Our main effects were species (*F. japonica, P. virginiana*) and treatment (50% shade vs. ambient light, hereafter called "shade" and "light"); population was nested within species (CM, MP, PG, and SB for *F. japonica*, BP, CM, CS, and UP for *P. virginiana*) and plot (1, 2, 3) was nested within treatment. We also included the following interaction terms: species by treatment, plot by species, and population by treatment. To examine the differences in trait means between the species and treatments, we used the absolute values of the residuals from ANCOVAs of each trait with a model that included the initial weight as a covariate.

Several traits (days until first shoot emergence, F_v/F_m, R:S, and specific leaf area) could not be transformed to meet the assumptions of normality for analysis, so we performed a nonparametric ANCOVA on the ranks of these data. Residuals from an analysis for each trait taking into account the covariate were used to test for significant differences between species and treatments using Kruskal-Wallis tests and chi-square approximations.

We also performed a multiple regression analysis of the parametric traits on final total biomass. This procedure mimics the phenotypic selection analysis developed by Lande and Arnold (1983), however our experiment was conducted in the controlled conditions of a greenhouse and thus removed any correlations between traits and fitness that are due to selection in a natural environment. Before the analysis, we checked traits for collinearity and found no significant correlations between the traits and therefore, none were removed from this analysis. In the context of natural selection in wild populations, Lande and Arnold (1983) applied regression analyses to determine what traits are under selection and how strong the selection is. We performed a linear regression of fitness on the measured traits, which produced coefficients (analogous to selection differentials (s) in a phenotypic selection analysis) that quantify the total effect that a trait has on fitness (that is, any relationship between that particular trait and fitness *plus* any effect on fitness due to that trait's correlations with other traits). Multiple regression of all traits on fitness produces partial regression coefficients (analogous to the selection gradients (ß) of Lande and Arnold (1983)), which quantify the direct effect of each trait on fitness while controlling for the effects of that trait's correlations with other traits. Relative fitness was calculated by dividing total biomass by the mean biomass for the treatment, and all other trait values were standardized by dividing them by their respective standard deviations for the particular treatment. Initial rhizome mass was added to all regression models as a covariate. Note that we used the untransformed total biomass as the fitness proxy for the selection analysis, as it is inappropriate to transform fitness values beyond making them relative (Lande and Arnold 1983).

Results

Results from the parametric nested analysis of covariance showed a significant (and not unexpected) effect of species, treatment, and population of origin on all traits (Table 2). Individual plots within each treatment were no different from each other, except with respect to leaf number. Means of absolute values of residuals from an ANCOVA of each trait with initial rhizome mass as a covariate are plotted in Figure 1; these adjusted means allowed us to examine what the trait values would be if initial rhizome mass were held constant. *P. virginiana* maintained a similar biomass and leaf area across the treatments, whereas *F. japonica* had greater biomass and leaf area in the light treatment (Figure 1a, 1d). *F. japonica* largely retained the same growth rate in the light and the shade, however the *P. virginiana* growth rate was greater in the light (Figure 1b). Differences in height and leaf number (Figure 1c, 1e) were only slight between treatments for each species; however, *P. virginiana* had greater mean values than *F. japonica* in both conditions. Both species showed an increase in stomatal density in the light (Figure 1f), again with *P. virginiana* having the higher value in both treatments.

The nonparametric ANCOVA showed a significant effect of population of origin for all traits and species for all traits except for R:S (Table 3). Kruskal-Wallis tests run on the rank distributions showed a difference in distribution for both species and treatments in F_v/F_m, R:S, and SLA (df=3, $\chi^2 > 60$). However, there was no difference between these groups for days until first shoot emergence, illustrating that differences observed during the experiment were largely driven by differences in initial rhizome weight (Figure 2).

Linear regression showed that most traits have a total effect on fitness, though to differing degrees (Table 4). The multiple regression analyses illustrated which traits had an influence on fitness when all other (measured) traits are held constant and whether or not this correlation changed across treatments. With the exception of growth rate in *P. virginiana*, all coefficients were positive (Table 4, Figure 3). For *P. virginiana*, there was little to no difference in the partial regression coefficients for all traits, indicating no change in the influence of the trait on fitness in different treatments. For *F. japonica*, however, leaf area, leaf number, and stomatal density all had a greater influence on fitness in the light treatment than in the shade treatment.

Discussion

Many have suggested the need to increase the evolutionary perspective in research on invasion biology (e.g., Lee 2002, Parker *et al.* 2003, Weinig 2007, and many others). Studies, like this one, that compare the fitness of species that are invasive and non-invasive seek to do just that. Baker proposed that an "ideal weed" would be phenotypically plastic in such a way as to be able to maintain its fitness in a variety of environments (Baker 1965, 1974). Here we compare an invasive species, *F. japonica*, with a native and non-invasive relative, *P. virginiana.* It is known that *F. japonica* is plastic for traits related to salt tolerance, thus aiding its invasion into new areas including salt marshes (Richards *et al.* 2008). We asked here whether, when compared to a noninvasive species, *F. japonica* is more plastic in traits related to light acquisition and how this plasticity might affect its fitness in different light regimes.

Overall responses of the traits to the different light regimes often followed what is expected from what we know about plant physiology. For example, stomata play an important role in the balance between photosynthetic rate and water loss and we should expect to see an increase in stomatal density in a higher light environment due to the corresponding increase in photosynthesis (Casson and Gray 2007). Our results did indeed show this; both species responded to the higher light treatment with an increase in stomatal density. However, *F. japonica* had a steeper reaction norm than *P. persicaria*, which showed only a very slight increase in stomatal density. After initial biomass was taken into account, we saw no difference between treatments for how long it took for the first shoot to emerge. Given that, necessarily, the shoots are not exposed to light before they have emerged, it makes sense that light treatment would have no effect on how long it took them to do this.

Leaf area remained the same in *P. virginiana* in the light and shade treatments and increased for *F. japonica* in the light. This result was surprising because neither of these is the expected response to light. Leaves grown in higher light conditions tend to be smaller because larger leaves have a harder time with heat transfer due to their greater evaporative demands and lower boundary layer conductance (Niinemets *et al.* 2007). In a study of a congeneric pair of native and invasive dandelions, increasing leaf area in shaded conditions is a selectively advantageous response (Brock *et al.* 2005). Since biomass in *F. japonica* increased in the light anyway, we cannot say that this increase in leaf area in the light treatment was detrimental to *F. japonica* or selected against in anyway. This finding is opposite that of the previous reciprocal transplant (O'Donnell 2010, Chapter 3), where both species had the expected decrease in leaf area in the lighter environment.

Results from the analysis of covariance showed a significant interaction between species and treatment for all parametric traits except leaf area, indicative of differences in plasticity traits between the two species. Overall performance (biomass) remained the same in both treatments for *P. virginiana* but increased in the light treatment for *F. japonica*. It should be noted that the increase in biomass in the light was not accompanied by an increase in growth rate, but this is most likely a result of our using changes in height alone as an estimate of growth. This increase in biomass suggested that, at least for *F. japonica*, the light treatment represented a higher quality environment. Using this finding, we were able to examine which traits that we measured contributed to this increase in fitness through our multiple regression analysis. Because we related measured traits to fitness, we can reach stronger conclusions regarding plasticity and its relationship to plant invasion (Richards *et al.* 2006).

For *F. japonica*, the observed plasticity in leaf area (although physiologically odd) is associated with a correlated increase in fitness with respect to that trait. The same pattern was seen with stomatal density, though, as we said above, this makes more sense with respect to what we know about plant physiology. In terms of leaf area, leaf number, and stomatal density, our results indicated that *F. japonica* was able to increase its fitness through the plastic responses of these traits. This finding suggests that, at least in terms of these traits, *F. japonica* is following the opportunistic, Master-of-some strategy. This species is able to take advantage of a higher quality environment through the plastic responses of some of its traits that are correlated with fitness.

We did not see this pattern with the traits for *P. virginiana* in the light and shade treatments. There was largely no difference in the partial regression coefficients that related the traits to fitness. Plasticity for growth rate and height in *P. virginiana*, but no change in the overall fitness or fitness relationships could be indicative of a broader tolerance to light regime differences. The plasticity displayed by *P. virginiana* allowed it to maintain a similar fitness level across the treatments. These results were the opposite of what was found in the reciprocal transplant (O'Donnell 2010, Chapter 3), in which *P. virginiana* was the Master-of-some and *F. japonica* was the Jack-of-all-trades. This conflict may be indicative of unmeasured environmental factors in the natural habitats having an effect on the plants responses to light.

With both the native and invasive species displaying phenotypic plasticity related to light quantity, our results parallel findings on other studies of invasive species. Brock *et al.* (2005) saw no difference in levels of plasticity between native and invasive *Taraxacum* species living sympatrically on a mountain. Each species displayed plasticity in several light related traits, but the specific traits sometimes differed (Brock *et al.* 2005). Muth and Pigliucci (2007) conducted an experiment with several species each of *Crepis* and *Centaurea* that varied in their levels of invasiveness and found that while the more invasive species tended to perform better in more environments (possessing the Jack-of-all-trades strategy), this response was driven by different traits with different relationships to fitness. These studies, as well as our results, preclude us from making any generalizations regarding whether or not an invasive species will have larger numbers of plastic traits than a native species.

It would be an interesting next step to examine selection on plasticity itself; perhaps it is the plasticity that is rapidly evolving and helping the invasive species invade. While we saw little evidence in terms of local adaptation (O'Donnell 2010, Chapter 3), indicating lack of rapid evolution in the specific traits we measured, the next step would be to look for selection on the reaction norms of the plastic traits. This experiment would require work done on plants in their native as well as invasive ranges, which would be especially interesting with *F. japonica* given that we know its invasion history and can also compare natural selection and response to selection in its two invasive ranges, Europe and North America. We know that *F. japonica* possesses little genetic variation in its invasive range (Richards *et al.* 2008) which makes trait plasticity more important in terms of how this plant can evolve in its introduced range.

Increased phenotypic plasticity, while certainly important, is not likely to be a perfect explanation for invasiveness. What is more likely is that plasticity in different traits is important at different stages of a plant invasion. From this standpoint, more work should be done on specific stages of invasion (initial colonization, for example) and how plasticity for certain traits interacts with plant fitness.

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Table 1: Locations of origin for plants used in the common garden experiment. All locations are in the state of New York on Long Island.

Abbreviation	*Location*	*Species*	*Average PAR ($\mu mol\ s^{-1}\ m^{-2}$)*	*Coordinates*
BP	Bethpage State Park, Bethpage	*P. virginiana*	24.09	N 40° 45.1’ W 73° 28.1’
CM	Caumsett State Park, Lloyd Neck	*P. virginiana* *F. japonica*	612.20 721.96	N 40° 55.0’ W 73° 28.6’ N 40° 56.2’ W 73° 27.3’
CS	Caleb Smith State Park, Smithtown	*P. virginiana*	55.98	N 40° 51.3’ W 73° 13.5’
MP	Mill Pond at Frank Melville Memorial Park, Setauket	*F. japonica*	99.56	N 40° 56.6’ W 73° 6.9’
PG	Paul T. Given County Park, Smithtown	*F. japonica*	560.99	N 40° 51.5’ W 73° 12.7’
SB	Baseball Field, Stony Brook University Campus	*F. japonica*	27.36	N 40° 55.4’ W 73° 7.4’
UP	Uplands Preserve, Cold Spring Harbor	*P. virginiana*	27.43	N 40° 51.3’ W 73° 27.2’

Table 2: Results from parametric analysis of covariance with initial weight as the covariate. * P≤0.05, **P≤0.01, ***P≤0.001

	Leaf Number	*Height*	*SQRT Growth Rate*	*SQRT Leaf Area*	*LOG Stomatal Density*	*LOG Total Biomass*
SPECIES						
df	1	1	1	1	1	1
MS	410.526773	7073.32202	2.11845857	299.907665	1.30911189	23.212803
F	**13.4491155*****	**30.8432987*****	**112.395521*****	**97.8576227*****	**65.5465259*****	**384.513982*****
TREATMENT						
df	1	1	1	1	1	1
MS	719.446073	860.138926	0.20967989	16.1029419	0.07475464	5.19717006
F	**23.5695063*****	**3.75064527***	**11.124636*****	**5.2542692***	**3.74292437***	**86.0897565*****
SPECIES x TREATMENT						
df	1	1	1	1	1	1
MS	162.952361	8904.40468	0.39910895	1.00368841	0.39735644	1.10230046
F	**5.33842193***	**38.8277548*****	**21.1748576*****	0.327496	**19.8954223*****	**18.2593175*****
POP[SPECIES]						
df	6	6	6	6	6	6
MS	291.930845	5021.11423	0.1495029	22.9024796	0.34622377	4.95211733
F	**9.56383829*****	**21.894624*****	**7.93192589*****	**7.47290739*****	**17.3352373*****	**82.0305224*****
PLOT[TREATMENT]						
df	4	4	4	4	4	4
MS	83.6387286	333.704417	0.02007896	5.60656315	0.00358289	0.0371195
F	**2.74005741***	1.45512179	1.06529579	1.82937952	0.17939313	0.61487477
POP x TREATMENT[SPECIES]						
df	6	6	6	6	6	6
MS	26.8667859	172.928647	0.03808683	3.0138007	0.03178088	0.10317992
F	0.88017282	0.75405727	2.02070939	0.98338057	1.59125126	1.70914823
PLOT x SPECIES[TREATMENT]						
df	4	4	4	4	4	4
MS	14.5028841	109.98698	0.02239863	1.84051291	0.01548173	0.09719609
F	0.47512361	0.47959944	1.18836663	0.60054556	0.77516179	1.61002763
INITIAL RHIZOME MASS						
df	1	1	1	1	1	1
MS	4374.00296	66353.6739	1.27498046	131.967382	0.21014967	33.3399659
F	**143.295091*****	**289.335927*****	**67.6445108*****	**43.0599674*****	**10.5220806*****	**552.267774*****

Table 3: Results from nonparametric analysis of covariance with initial weight as the covariate using ranked values for each trait. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

	Ranked Fv/Fm	*Ranked R:S*	*Ranked SLA*	*Ranked Days until Emergence*
SPECIES				
df	1	1	1	1
MS	542142.911	122.671357	1547049.44	9856134.01
F	**30.1626645*** **	0.00438873	**135.817005*** **	**661.909644*** **
TREATMENT				
df	1	1	1	1
MS	27761.2137	631980.74	2190856.11	18797.1661
F	1.54452297	**22.6099708*** **	**192.337431*** **	1.26236367
SPECIES x TREATMENT				
df	1	1	1	1
MS	33606.6753	831776.68	10636.354	42501.1638
F Ratio	1.86974108	**29.7579423*** **	0.93377607	2.85425606
POP[SPECIES]				
df	6	6	6	6
M	183263.589	650900.685	96635.8651	183403.545
F	**10.1960536*** **	**23.2868575*** **	**8.48375846*** **	**12.3168552*** **
PLOT[TREATMENT]				
df	4	4	4	4
MS	543373.981	51785.3333	136811.92	11020.7548
F	**30.2311564*** **	1.85269075	**12.0108541*** **	0.74012223
POP x TREATMENT[SPECIES]				
df	6	6	6	6
MS	26757.2055	49804.4514	17087.5857	30641.9396
F	1.48866396	1.78182201	1.5001361	2.05782463
PLOT x SPECIES[TREATMENT]				
df	4	4	4	4
MS	433927.63	34824.89	79783.6642	6383.16635
F	**24.1419989*** **	1.24590782	**7.00428702*** **	0.42867511
INITIAL RHIZOME MASS				
df	1	1	1	1
MS	11.2865592	1007131.47	144107.788	66862.9752
F	0.00062794	**36.0314986*** **	**12.6513656*** **	**4.49032532**

Table 4: Results from linear and multiple regressions of fitness (total biomass) on standardized trait values. Means reported were calculated from untransformed and unstandardized data. * $P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

	Species	*Mean (untransformed)*	*Std. Regression Coefficients from Linear Regression*		*Std. Partial Regression Coefficients from Multiple Regression*	
Height						
Shade	*F. japonica*	48.86	**0.45**	***	**0.28**	***
	P. virginiana	23.31	**0.30**	***	**0.36**	***
Light	*F. japonica*	41.52	**0.54**	***	**0.23**	***
	P. virginiana	34.46	**0.23**	***	**0.20**	***
LOG10 Stomatal Density						
Shade	*F. japonica*	73.59	**0.08**	*	0.05	
	P. virginiana	98.34	0.05		0.03	
Light	*F. japonica*	65.59	0.13		**0.13**	*
	P. virginiana	124.88	0.00		0.03	
SQRT Growth Rate						
Shade	*F. japonica*	0.14	**0.24**	***	**0.08**	*
	P. virginiana	0.26	**0.06**	*	**-0.11**	***
Light	*F. japonica*	0.13	**0.38**	***	0.04	
	P. virginiana	0.40	0.04		**-0.09**	**
SQRT Leaf Number						
Shade	*F. japonica*	13.55	**0.29**	***	**0.20**	***
	P. virginiana	8.14	**0.20**	***	**0.23**	***
Light	*F. japonica*	14.81	**0.42**	***	**0.37**	***
	P. virginiana	11.80	**0.20**	***	**0.16**	***
SQRT Leaf Area						
Shade	*F. japonica*	37.41	**0.29**	***	**0.20**	***
	P. virginiana	16.23	**0.22**	***	**0.11**	**
Light	*F. japonica*	42.98	**0.36**	***	**0.39**	***
	P. virginiana	17.39	**0.13**	***	**0.08**	**

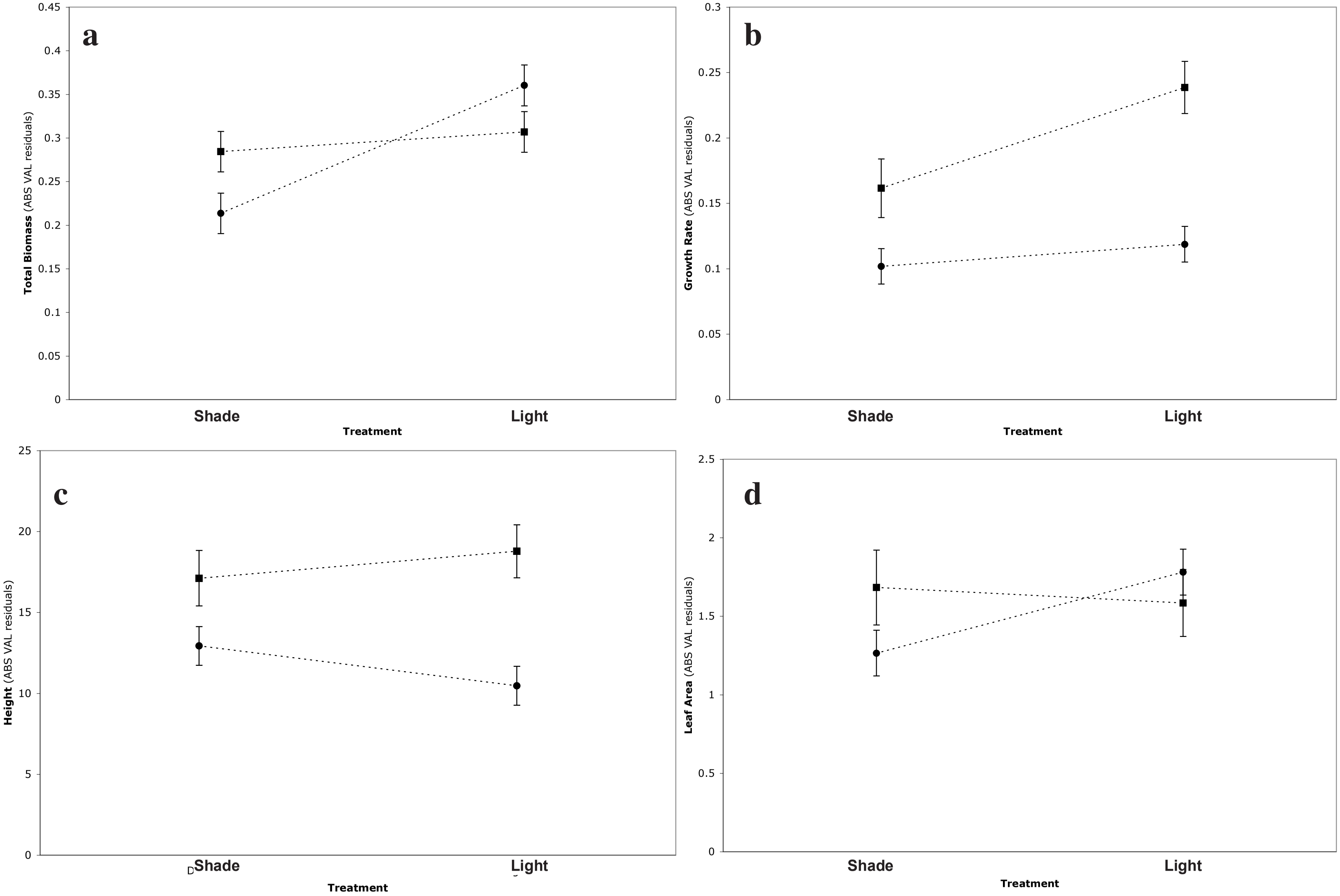

Figure 1(a-d): Adjusted means of traits calculated from the absolute values of residuals of an analysis of covariance using initial rhizome mass as the covariate. Circles represent *F. japonica* and squares represent *P. virginiana*. Error bars are 95% confidence intervals.

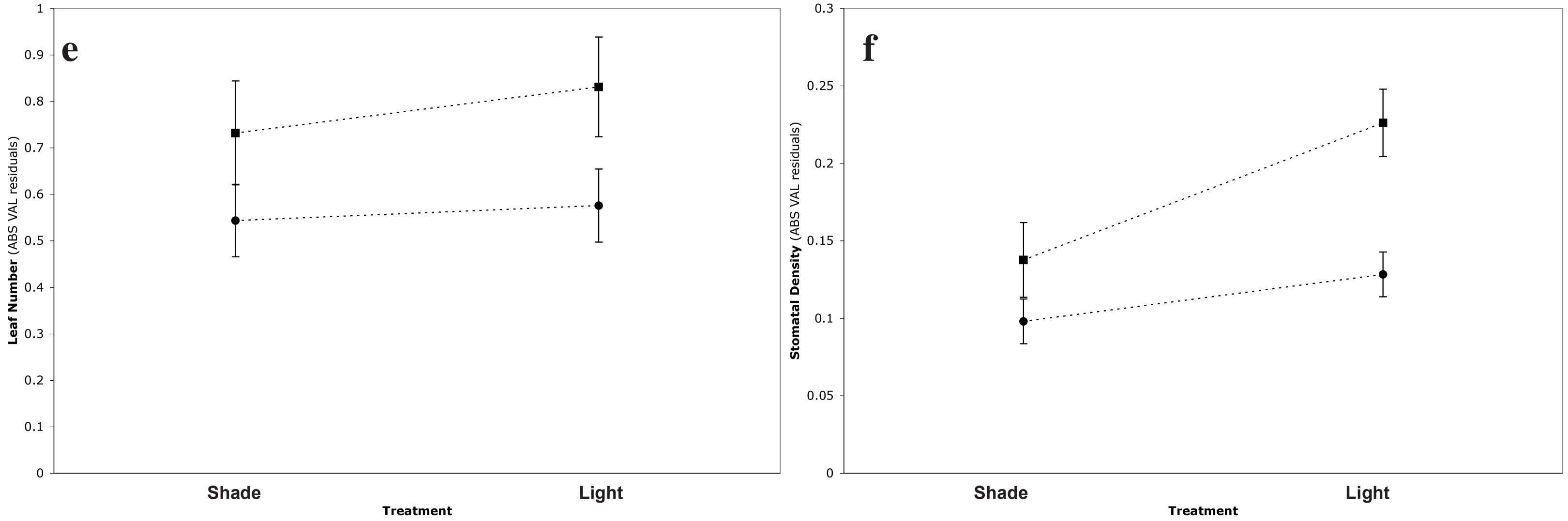

Figure 1(e-f): Adjusted means of traits calculated from the absolute values of residuals of an analysis of covariance using initial rhizome mass as the covariate. Circles represent *F. japonica* and squares represent *P. virginiana*. Error bars are 95% confidence intervals.

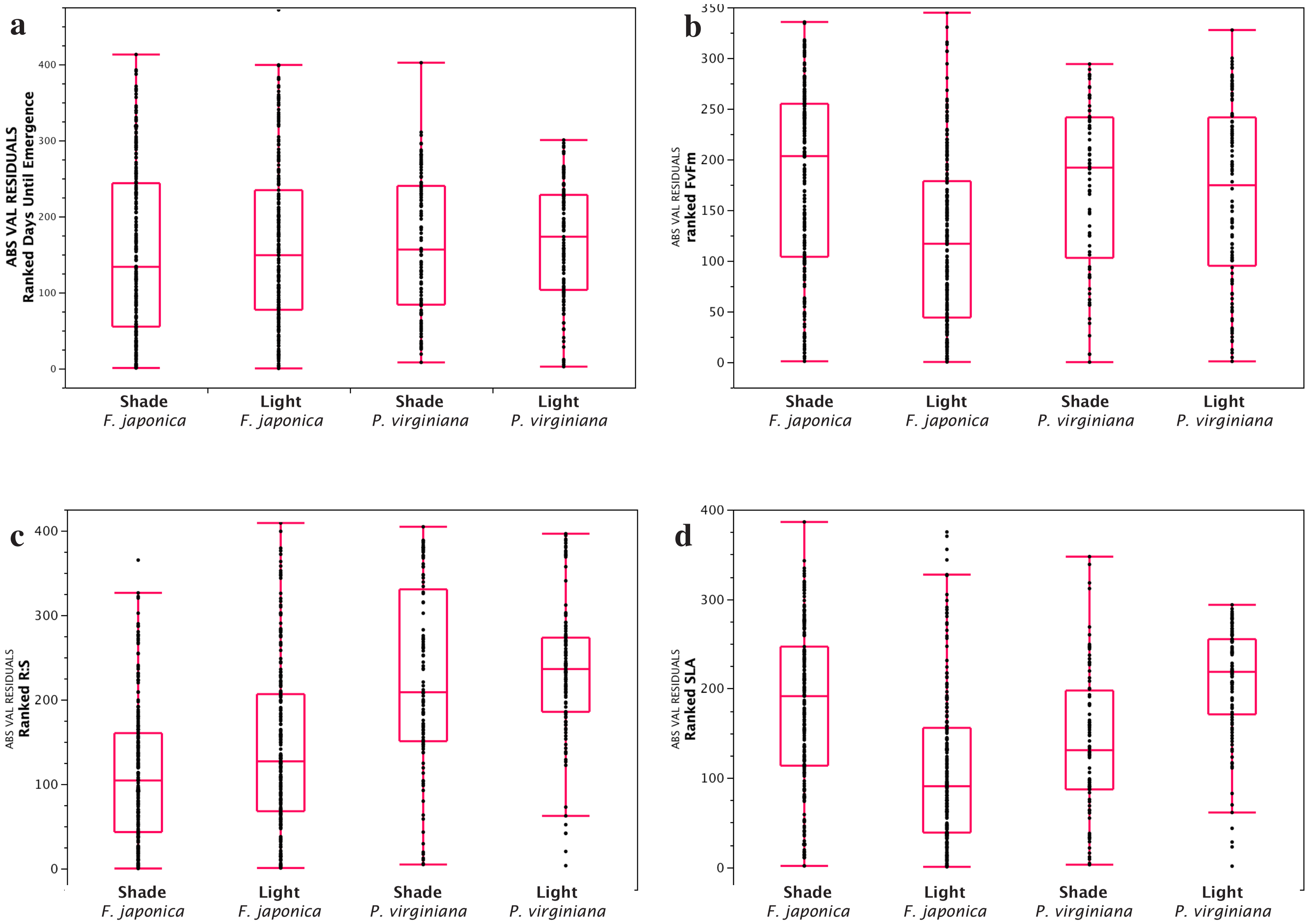

Figure 2: Box plots of nonparametric traits from each species in each trait. The ends of the boxes designate the 25% and 75% quantiles. Lines inside the boxes indicate the medians. Whiskers enclose the rest of the distribution of datapoints.

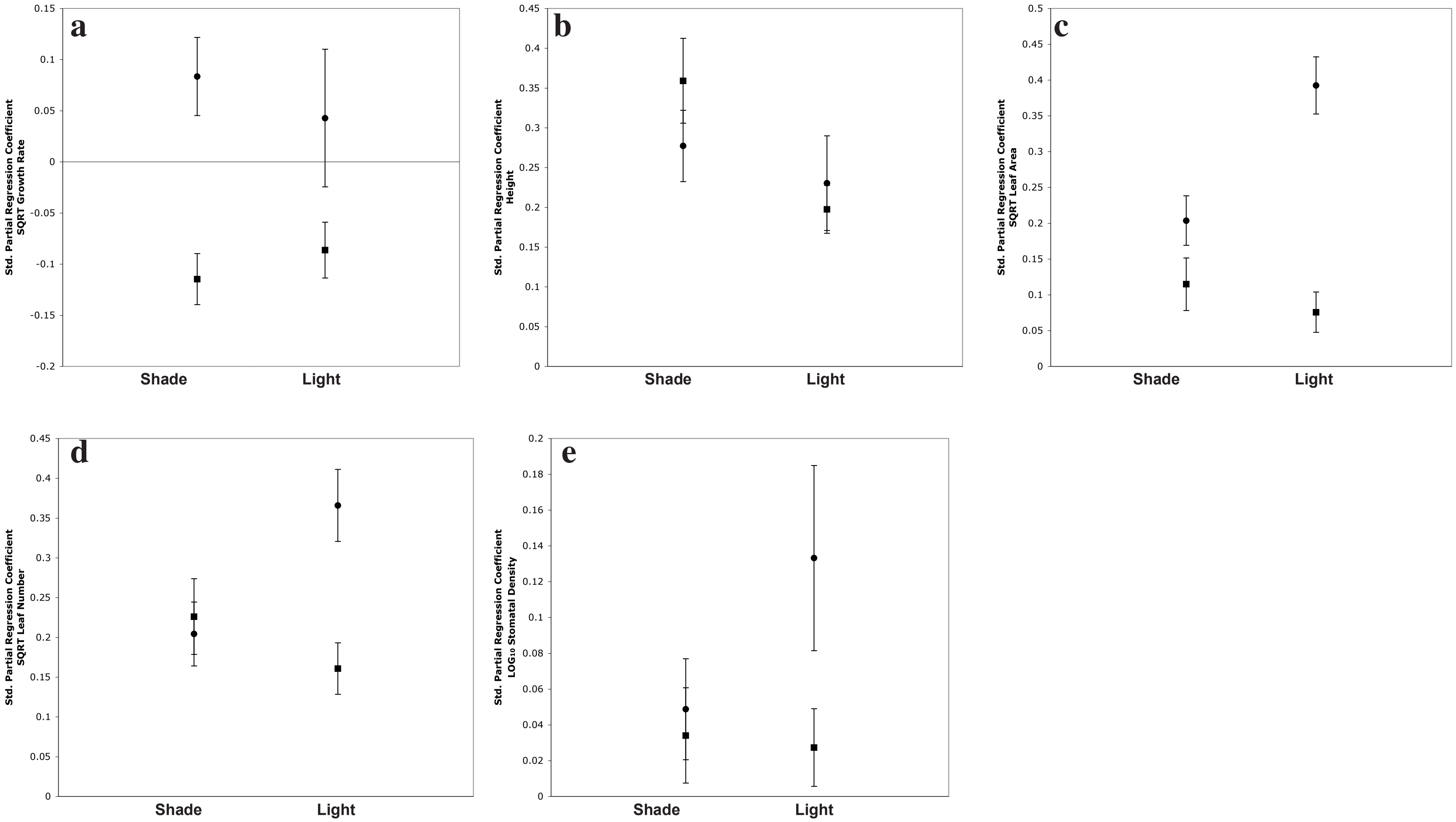

Figure 3 (a-e) - Standardized partial regression coefficients from a multiple regression of five traits on biomass. These represent which traits are highly correlated with fitness (biomass) when variation due to other traits is accounted for. Circles represent *F. japonica*, squares respresent *P. virginiana*, and bars represent ± one standard error.

Conclusions

The colonization of new habitats by invasive species represents an excellent opportunity to examine the dynamics of natural selection in the wild. These invaders must respond to potentially novel conditions to survive, whether that response is adapting to the new conditions or adjusting through plastic traits. In Chapters 1 and 2, we demonstrated not only that natural selection is detectable in the field, but also that it is strong. There was no difference in the magnitude of selection attributable to status as a native or invasive in either analysis. The most striking result from the selection analyses conducted on *F. japonica* and *P. virginiana* (Chapter 2) was the highly variable nature of the magnitude and direction of the selection gradients across the three years of this study. These results are consistent with the findings of Siepielski *et al*. (2009), who reviewed multi-year selection analyses (of which there are not many) and observed a wide range of variation in strength and total variation among years of a given study. They suggest that perhaps the common question of whether selection is strong or weak in nature would be better put in a context of annual variation in selection strength and how populations respond to fluctuations of both the magnitude and direction of selection (Siepielski *et al.* 2009). Temporal fluctuations in selection strength and direction have important implications for investigations of rapid or so-called contemporary evolution. We would expect change to occur more rapidly with consistent selection gradients. However, without the ability to constantly monitor selection pressures in wild populations, often due to lack of a large workforce and funding, how will we know if we have missed a period of rapid evolution as a result of natural selection? A notable example of the kinds of information needed to create a more thorough picture of the selection dynamics of a population is Grant and Grant’s (2002) thirty-year analysis on selection that contained pedigree data, environmental conditions, and selection estimates. Unfortunately, this kind of study, with its vast data series, is rare and will continue to be so given that funding agencies seem disinclined to fund this sort of research. They most likely assume that because natural selection is such a fundamental tenet of evolutionary biology, there is no more need to pursue answers to basic questions regarding selection in nature. However, this is not the case as shown by the results here and by the many who have lamented about it previously (see Kingsolver *et al.* 2001, Hendry 2005, Siepielski *et al.* 2009).

Regardless of the consistency in strength or direction, we did detect very strong selection gradients. Could these have molded the populations as they are today? This question was the basis for Chapter 3. The analysis showed very little local adaptation to light condition, if any at all. In most cases, plants did not perform any better in their home site compared to the site into which they were transplanted, regardless of species. If we look specifically at the effect of the different light regimes on biomass, which was used in a corresponding selection analysis as a fitness proxy, we see both transplants and replants of *P. virginiana* had increased in biomass (almost to the same degree) in the light environment, with similar biomass despite different light regime in *F. japonica.* This is suggestive of an opportunistic strategy in *P. virginiana* in which the plants are not locally adapted, but can exploit a change to a better quality environment. *F. japonica*, also showing no local adaptation, did show a robustness that allowed it to maintain its fitness regardless of where it was planted. Both of these findings suggested a role for phenotypic plasticity in the survival and perhaps evolution of these populations.

The goal of the common garden experiment in Chapter 4 was to further examine plasticity in traits related to light acquisition, which are likely to have an effect on plant fitness. There was significant interaction between species and treatment for all parametric traits except leaf area, indicative of differences in plasticities between the two species. Overall performance (biomass) remained the same in both treatments for *P. virginiana* but increased in the light treatment for *F. japonica*. This result was the opposite of what was found in the reciprocal transplant, which may be indicative of unmeasured environmental factors in the natural habitats having an effect on the plants responses to light. *F. japonica* was able to increase its fitness through the plastic responses of leaf area, leaf number, and stomatal density. Using the strategy framework set forth by Richards *et al.* (2006), our findings suggested that, at least in terms of the traits measured, *F. japonica* is following the opportunistic, Master-of-some strategy. Plasticity for growth rate and height in *P. virginiana*, but no change in the overall fitness or fitness relationships to these traits, could be indicative of a broader tolerance to light regime differences. The plasticity displayed by *P. virginiana* allowed it to maintain a similar fitness level across the treatments.

Of course, there is much left to be done on this invasive plant that could elucidate the relationship between phenotypic plasticity and invasiveness. One interesting road to pursue would be to look for selection on the reaction norms of the plastic traits. Is there evolution for an increase in the plasticity of ecologically relevant traits in the invasive plant? This experiment would require work done on plants in their native range as well as their invasive range, which would be especially interesting with *F. japonica,* given that we know its invasion history and can also compare natural selection and response in its two invasive ranges, Europe and North America. Increased phenotypic plasticity, while certainly important, is not likely to be a perfect explanation for invasiveness. What is more likely is that plasticity in different traits is important at different stages of a plant invasion. From this standpoint, more work should be done on specific stages of invasion (initial colonization, for example) and how plasticity for certain traits interacts with plant fitness.

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