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## Phenotypic Variation in Native North American and Invasive Chinese Populations of *Plantago Virginica*

Teresa E. Popp

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PHENOTYPIC VARIATION IN NATIVE NORTH AMERICAN AND INVASIVE CHINESE  
POPULATIONS OF *PLANTAGO VIRGINICA*

by

TERESA POPP

Under the Direction of Lorne Wolfe

**ABSTRACT**

Alien plant species can cause significant economic and biological destruction by invading new environments and outcompeting native species. Through experimental research, the mechanisms by which these species invade can be better understood and applied to their management. *Plantago virginica* is a perennial herb, native to North America that has recently invaded China. It has been known to reduce crop growth and harbor diseases in the introduced range. A common garden study was conducted at the Georgia Southern University greenhouse, comparing twenty populations of *P. virginica* from both the native North American and invasive Chinese ranges. I examined if there were genetically based differences in a suite of life history, morphological and reproductive traits between populations from both the native and introduced range. Overall there were significant differences in traits among populations within regions. Specifically, Chinese populations germinated sooner, but produced reproductive structures and seeds later than their North American counterparts. Also, introduced populations had less phenotypic diversity in several traits compared the native populations. These patterns are consistent with the effects of natural selection, however, the role of random genetic drift cannot be ruled out. They also suggest that post-introduction phenotypic evolution has occurred in Chinese populations of *P. virginica*, contributing to its invasiveness.

**INDEX WORDS:** Plant invasion, Common garden, *Plantago virginica*, Competition, Phenotypic variation, Evolved Life History Traits

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## CHAPTER I

### INTRODUCTION

*“..... for in all countries, the natives have been so far conquered by naturalised productions, that they have allowed foreigners to take firm possession of the land. And as foreigners have thus everywhere beaten some of the natives, we may safely conclude that the natives might have been modified with advantage, so as to have better resisted such intruders.” (Darwin 1859)*

An invasive species is an organism occupying a region in which it was not previously present, but has been able to develop self-sustaining populations at the expense of the native species (Sax et al. 2005). These organisms are able to successfully establish by either (1) undergoing a genetic change to become a more aggressive colonizer, (2) escaping the attention of its natural pests and competitors (enemy release hypothesis), or (3) finding an unoccupied niche (Baker 1991). As the invader fills an empty niche within the new ecosystem, it may be able to acquire resources more easily than native species making these resources less available for the surrounding organisms (Mitchell et al. 2006). The invader could take over the new range and possibly change the ecological dynamics of the entire area, thus becoming a threat to the native biodiversity (Sax et al. 2005). Regardless of how the organism was able to establish, the toll of these invasive species is estimated to range from millions to billions of dollars annually (Sakai et al. 2000, Suarez et al. 2008, Pimentel et al. 2000). Beyond these economic damages, invasive species are responsible for severe ecological damage (Ludsin & Wolfe 2001) resulting from competition, predation, and hybridization, which can cause the transformation of populations and communities (Suarez et al. 2008).

The evolutionary success of organismal invasion is influenced by the attributes of the invader, biotic characteristics of the invasive site, and the environmental characteristics of the invaded area (Perkins et al. 2011). The evolution of an invasive species occurs in three steps. First, the species needs to be introduced in to a novel environment, which poses selection pressures that were not

present in its native range. Second, if the species is able to establish, it may be selected upon by key ecological forces that impact its performance. Finally, after the successful traits have been selected, evolutionary adjustments can be made to fit the novel environment (Bossdorf et al. 2004).

Baker (1965) coined the term “general purpose genotype” in order to describe colonizing species that thrive in a wide range of environmental conditions through phenotypic plasticity (Baker & Stebbins 1965). If these phenotypic traits affect the reproductive success of individuals, the next generation will be impacted. Although no set of traits has been found to be the same in every invasive species, there are factors that are known to promote the success of an invasion. Plants in particular are able to reproduce both sexually and asexually, rapidly grow from seedling to sexual maturity, endure environmental stress, and have a high tolerance to environmental heterogeneity (Sakai et al. 2001). A recent study looking at phenotypic plasticity in fitness-related traits, found that this plasticity promoted invasiveness in the native Mediterranean forb *Centaurea melitensis* by enhancing the plant’s competitive ability and reproductive success in the invaded California range. The non-native *C. melitensis* was able to flower earlier and grow faster in early growth stages, attributing to its invasiveness (Moroney et al. 2013). Understanding the traits of a species of interest may allow researchers to better understand the forces underlying an invasion, uncover a defense against them, or learn how to protect the biodiversity in uninvaded areas.

Flowering plants display a unique range in the reproductive systems (Baker 1955, Barrett et al. 2008). They are able to reproduce sexually through pollen dispersion among unrelated individuals (outcrossing), through crossing pollen within a single plant (self-fertilization or selfing) or produce a mixed mating system (Barrett et al. 2008). Selfing plants have an advantage over outcrossing plants in that they do not need to rely on pollinators to ensure offspring production (Baker 1955). However, the offspring of outcrossing plants have a higher probability of survival because of high levels of genetic variation and are not subject to inbreeding depression (Barrett et al. 2008, Albert et al. 2011). One type of specialized selfing is cleistogamy, the production of permanently closed flowers (Culley & Klooster 2007). Cleistogamous flowers are less costly to produce than open flowers because they are

smaller and the plant no longer needs to rely on the presence of pollinators and allows both sets of maternal genes to be passed on to the offspring (Culley & Klooster 2007). These selfing mechanisms are believed to be favored in population establishment (Barrett et al. 2008), more commonly known as Baker's Law (Stebbins 1957). The reproductive assurance provided by selfing can allow the establishment of an invasive plant species, even if the founding group is small in size. Theoretically, a single individual could initiate the establishment of the species through self-fertilization (Barrett et al. 2008). However, this type of reproductive system can lead to populations losing genetic diversity in the invasion process through genetic bottlenecks and restrictions on genetic recombination (Waller 1984). This is why many large-scale invasions are thought to have occurred through multiple introductions as well as by plants with mixed mating systems (Barrett et al. 2008).

The ecological forces involved in an invasion can be studied by comparing the invasive species to its native counterpart. This includes determining the phenotypic and genetic differences of these once genetically identical organisms, determining why it becomes more successful in a novel range, and what makes it problematic for the new ecosystem it has invaded (Wolfe & Blair 2009). One of the best methods to study invasive species is through the use of a common garden experiment, where several populations of the same species from both the native and invaded range are grown under controlled environmental conditions, and are measured for phenotypic differences. This allows researchers to determine whether observed differences among field populations have a genetic basis (Clausen et al. 1948, Parker et al. 2002, Blair & Wolfe 2007). Strickler (2013) found that when growing native and invasive species of the shrub *Eugenia uniflora* in a common garden study, invasive seedlings outperformed their native counterpart. This study also suggests that because of having more robust seedlings, these invasive populations may possess a competitive advantage. Another study compared the morphological traits in 23 species, using herbarium specimens, from their native and invasive ranges approximately 150 years post introduction (Buswell 2011). They showed that 70% of the species exhibited a change in at least one trait through time, suggesting that rapid evolution of introduced plant species could be much more common in plant invasion than

previously thought. Daehler (2003) looked at the factors that make some plant species troublesome invaders and found that few invaders had universal advantages over naturally occurring species; but were more successful in their invasion when their introduction was associated with increased resource availability or altered disturbance regimes due to human activity (Daehler 2003). Although Daehler (2003) showed that competitive ability with naturally occurring species may not be as influential in a species invasion as previously thought, the idea of the enemy release hypothesis could still be the key factor to some invasions. This idea of enemy release suggests that the new range allows for the introduced species to “escape” from their natural predators present in the native range (Genton et al. 2005). This also can be described as reduced top-down control, meaning energy that was once allocated toward defenses can now be redistributed toward growth and reproduction. If this occurs, the evolution of increased competitive ability (EICA) can result in the enhanced performance of an organism compared to the native plant populations (Blossey & Notzold 1995, Wolfe 2002, Wolfe & Blair 2009), allowing it to express enhanced performance.

The competitive ability of an invasive species can be understood by growing the species of interest in the presence of a second species; allowing genotypic traits to be attributed to the presence of the competition. Goldberg and Landa (1991) proposed that individual competitive ability can be compared between regions in two different ways: in their competitive effect on other individuals, and their competitive response in avoiding being suppressed by other individuals (Goldberg 1991). When considering the role of an invasive species and its competitive interactions, populations from both the native and invasive range need to be grown in the presence and absence of second species and well as growing the second species in the presence and absence of competitor. It is important to consider how the two species interact and how the native and invasive populations differ to determine an invasive’s dominance and persistence in natural plant communities around the world.

### Thesis Research and Justification

Biological invasions have become a global challenge. The study and management of invasive species is not limited to single regions; they are able to spread rapidly to previously uninhabited areas and have broad ecological consequences. International collaborations are particularly useful. This study had collaboration with China through the Fudan University in Shanghai, running parallel common garden experiments using *Plantago virginica* as a model. Joint research such as this can have significant academic benefits as well as enhancing the quality of scientific research.

The overarching goal of this research is to compare genetically-based differences in the herbaceous annual *Plantago virginica* between populations from the native range of North America and populations from the invaded range of China. Introduced to China 50 years ago, *P. virginica* has spread over the entire country and into parts of northern Taiwan (Yan et al. 2000). Using an experimental approach, I addressed the following questions:

- (1) Are there differences in morphological traits between the North American and Chinese populations?
- (2) Are there differences in competitive ability between North American and Chinese populations?

Comparing the growth and reproductive traits of different populations will aid in understanding the physical attributes that may have evolved in Chinese populations to make this plant an aggressive invader. When groups from different regions are grown under uniform environmental conditions, differences that exist in life history traits can be assumed to be genetically-based (Clausen et al. 1948). Using a common garden approach, I tested the hypothesis that Chinese populations of *P. virginica* have evolved life history traits that have allowed the plant to become a robust invader compared to its North American counterparts. A second greenhouse study was conducted to test the hypothesis that Chinese populations of *P. virginica* have evolved to become more aggressive competitors than their North American counterparts (i.e. have greater plant biomass). If there has been evolution in the Chinese ranges, the process by which they outcompete native species can be better understood.

## CHAPTER II

### MATERIALS AND METHODS

#### Study Species and Source Material

The genus *Plantago* (Plantaginaceae) is distributed worldwide and consists of over 200 species of both perennial and annual herbs and subshrubs (Ronsted et al. 2002, Dunbar-Co et al. 2008). This dicotyledon genus is wind-pollinated and common to the eastern half of the United States (Poot 1997, Radford et al. 1968) found in grasslands and along roadsides (Cui et al. 2011). *Plantago* exhibits dimorphic cleistogamy, in that it produces both open and cleistogamous (closed) flowers (Albert et al. 2011, Barton 2007) arranged as spikes on inflorescences (Cui et al. 2011). It has been the focus of a number of studies examining the plasticity of phenotypic traits. Research suggests that the energy allocation by *Plantago* is almost exclusively to seed production (Primack 1979). Others have demonstrated differences in physiological traits in response to changes in environmental factors (Schlichting 1986); for example, it is tolerant of low temperatures, low nutrient availability, high salt concentrations, and high concentrations of heavy metals (Hoggard 1998).

Both *Plantago lanceolata* and *Plantago major* are among the top ten most problematic weeds in the world (Hoggard 1998) and have been shown to reduce crop growth and harbor diseases (Hoggard 1998). Several *Plantago* species have been found invading new regions. *Plantago virginica*, for example, is native to North America but has invaded parts of China (Yan et al. 2000) and has been able to spread rapidly due to its prolific seed production. First collected in Nanchang (Jiangsu Province) in 1951, *P. virginica* now occurs in the grasslands and roadsides of the Chinese provinces of Jiangsu, Anhui, Zhejiang, Fujian and parts of northern Taiwan (Yan et al. 2000). This plant is now being studied to help understand why it is such a successful invader, and perhaps to develop a potential defense against it in uninvaded areas such as Korea and Southern Taiwan.

### **Common Garden Experiment**

To determine if the life history and reproductive traits of *P. virginica* have evolved in China compared to plants in its native range in the United States, plant performance was compared using a common garden approach. Genetic factors were quantified through the comparison of phenotypic and life history traits of native and invasive populations by growing numerous genotypes under homogeneous conditions (Moloney et al. 2009). This common garden experiment was conducted in the greenhouse at Georgia Southern University (Bulloch County, GA) in the spring of 2013.

*P. virginica* seeds were previously collected by Dr. Hui Guo (Fudan University, Shanghai, China) and Dr. Lorne M. Wolfe, including ten populations from across the native North American and ten from the invasive Chinese range (Table 1, Fig 1). Twenty individual seeds from each population were germinated ( $20 \times 10 \times 2 = 400$ ) on moistened filter paper in a petri dish, allowing for germination to be controlled for environmental influences to ensure germination success was due to genetic factors. After appearance of the first leaf, twenty seedlings from each population were transferred to individual containers (radius= 2cm, length= 13.5cm) filled with standard soil mix (Miracle Grow: moisture control potting mix). Containers were labeled with respect to population and continent and randomly arranged on the greenhouse benches. Plants were monitored for growth and watered as needed.

#### ***Phenotypic and Life History Traits Measured***

The following traits were measured to test for genetically based differences between the native and invasive regions. Germination traits were measured to understand if the invader is better at establishing once introduced, while morphological traits show if the invader is able to allocate a greater amount of energy to growth and maintenance. Reproductive traits indicate if the invader has been able to put energy into the production of the next generation, which could contribute to its invasion success.

##### **(i) Germination Traits**

- a. Time to Germination- a plant was considered germinated when a cotyledon appeared.

- b. Germination Success- the fraction of the initial 20 seeds per population that germinated.

(ii) Morphological Traits

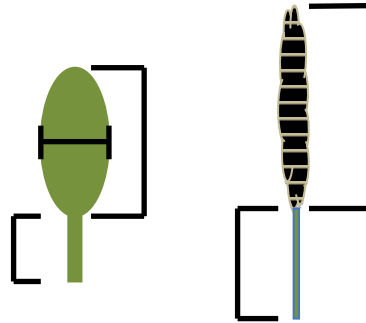
- a. Time to First True Leaf Emergence- the time it took for the first leaf to fully expand.
- b. Plant Size (measured as leaf number)- recorded approximately five weeks after FTL emergence and again two weeks after inflorescence emergence (before seeds developed).
- c. Leaf Size- one leaf from every individual was collected (five weeks after FTL emergence) by clipping the leaf at the plant stem. I measured the following traits:
  - i. Length (from leaf base to top of leaf)
  - ii. Petiole length (from plant stem to leaf base)
  - iii. Width (widest part of the leaf)
  - iv. Total leaf length (leaf length + petiole length)
  - v. Dry biomass
- d. Specific Leaf Area (SLA)- ( $SLA = \frac{\pi \times l \times w}{leaf\ biomass}$ ), where  $l$ = length of leaf not including the petiole (Delph 2001).
- e. Total Plant Area ( $(\pi \times l \times w) \times$  leaf number before inflorescence emergence)

(iii) Reproductive traits

- a. Time to Inflorescence Emergence- time inflorescence buds appeared at the base of the plant
- b. Time to Seed Maturation- (see appendix A)
- c. Number of Infructescences -The flowers on 25 infructescences were counted and the length of the infructescence was measured, suggesting that the number of flowers on an infructescence directly correlated with the length of that infructescence ( $R^2=0.94$ ,  $P<0.0001$ ) (Fig 2).
- d. Infructescence Size - I measured the following on each infructescence
  - i. Peduncle length



- ii. Infructescence length
- iii. Total Infructescence Length per plant (number of infructescences x infructescence length)
- e. Number of Cleistogamous and Open Flowers per Infructescence (see appendix B)



### Competition Experiment

Growing *P. virginica* from both the native and invasive regions in the presence of a second species (inter-specific competition), allows differences in life history traits to be contributed to the presence or absence of competition. Populations of *P. virginica* were grown in the presence of *Cynodon dactylon* (Bermuda grass), which is a species frequently used as lawn and garden grass seed in North America. A second Chinese competitor was not included in this study because, regardless of competitor's origin, the two species grown together were competing for the same nutrients and water.

A preliminary experiment was conducted to determine the optimal density of Bermuda grass to plant with the *P. virginica*. In 2"x2" pots 0.5g, 1g, 2g, 3g and 4g of Bermuda grass seed were planted. After growth, the pot containing 0.5g of grass seed was considered to contain a similar coverage of grass that *P. virginica* would encounter in natural conditions within a lawn. Also, at higher densities, the grass was to unable survive because they were competing with themselves for resources and were over the carrying capacity of the pot.

*Plantago virginica* seeds were germinated in Fall 2013. Twenty seeds from each *P. virginica* population were germinated on moistened filter paper in petri dishes (20 x 10 x 2 = 400). Once

germinated, fifteen seedlings from each population were transplanted (n=300) to individual 2”x 2” pots filled with standard soil mix (Jungle Growth). After the seedlings established (evident after emergence of a first true leaf), 0.5g of *C. dactylon* was added to the pots of ten individuals from each population (n=200). The remaining plants served as controls growing in the absence of competition (n=100). Plants were watered and monitored for growth for approximately 26 weeks and ended when infructescence growth ceased.

The effect of competition was measured by measuring and comparing the above ground dry biomass of *P. virginica* (excluding the biomass of *C. dactylon*). The more conventional approach proposed by Goldberg and Landa (1991) was not performed because measurements with the absence of competition were only acquired for *P. virginica*, *C. dactylon* was not grown in the absence of competition.

### **Data Analysis**

Statistical analysis was conducted using the statistical package JMP Pro (SAS Institute Inc., version 10, 2012). All data was tested for normality and homogeneity of variance and is presented as non-transformed means  $\pm$  standard error and the term *region* refers to North America vs. China. An ANOVA was used in the common garden experiment to determine if there were significant differences in the germination traits between regions, while a nested ANOVA was used to identify differences in morphological and reproductive traits. (General Model: Plant Trait = Region + Population (Region) + Error). Correlation analysis was conducted to determine the association between traits within each region. The coefficient of variance was calculated for traits within populations and then compared between regions using a t-test to determine overall the genetic variance between regions. A 2-way nested ANOVA was conducted in the competition experiment to determine if there were significant differences in above ground biomass among North American and Chinese populations in the presence or absence of competition (General Model: Biomass = Region + Population (Region) + Competition + Region\*Competition + Error).

## CHAPTER III

### RESULTS

#### Common Garden Experiment

##### *Germination Traits*

Significant regional differences were detected for one germination trait. Chinese populations of *P. virginica* had a 25% greater germination success than North American populations ( $P=0.018$ ; Table 2, Fig 3). Although not statistically significant, there was a trend of faster germination in Chinese populations ( $P=0.089$ ; Table 2, Fig 3).

##### *Morphological Traits*

Several morphological traits were found to be statistically different between the two regions. Chinese populations tended to produce their first true leaf faster than their North American counterparts ( $P=0.062$ ; Table 3, Fig 4). The regions did not differ in plant size (number of leaves) ( $P=0.18$ ; Table 3, Fig 4). North American plants tended to have longer leaves ( $P=0.067$ ) and significantly longer petioles ( $P=0.004$ ), however, Chinese populations had significantly wider leaves ( $P=0.033$ ; Table 3, Fig 4). North American leaves had a total length (including petiole) an average of 3mm longer than Chinese plants ( $P=0.010$ ), but did not differ in biomass ( $P=0.822$ ; Table 3, Fig 4). There was no difference between regions in specific leaf area (SLA) ( $P=0.99$ ) or total plant area ( $P=0.36$ ; Table 3, Fig 4).

##### *Reproductive Traits*

Significant regional differences were detected for several reproductive traits. Chinese populations of *P. virginica* took 14 days longer to produce reproductive structures (inflorescences) than North American populations ( $P<0.0001$ ; Table 4, Fig 5). North American populations produced seeds approximately 21 days earlier than Chinese populations ( $P<0.0001$ ; Table 4, Fig 5) and had a greater number of infructescences ( $P=0.003$ ; Table 4, Fig 5). Chinese populations, however, produced

68% higher infructescences (peduncle length) ( $P < 0.0001$ ), and 38% longer infructescences ( $P < 0.0001$ ), but had no difference in total infructescence length per plant ( $P = 0.858$ ; Table 4, Fig 5).

No chasmogamous flowers were produced in this study. Every inflorescence produced only cleistogamous flowers.

#### *Correlation and Coefficient of Variation Relationships*

In North American populations of *P. virginica*, the time to germination and the proportion of seeds germinated were correlated ( $R = -0.658$ ,  $P = 0.050$ ) as well as the time to inflorescence production and the length of the infructescence ( $R = 0.793$ ,  $P = 0.011$ ) (Table 5). In Chinese populations, the time to inflorescence production was related to the number of inflorescences a plant produced ( $R = -0.688$ ,  $P = 0.028$ ) (Table 6). In both North American and Chinese populations, the size of the plant and the time to inflorescence production were correlated ( $R = 0.718, 0.805$ ,  $P = 0.029, 0.005$ ) as well as the length of infructescences and how many infructescences a plant produced ( $R = -0.75, -0.69$ ,  $P = 0.019, 0.025$ ) (Table 5,6).

The coefficient of variation among traits in Chinese ( $0.157 \pm 0.033$ ) populations was lower than those of their North American ( $0.289 \pm 0.042$ ) counterparts ( $F_{(1,8)} = 6.13$ ,  $P = 0.027$ ; Fig 6).

#### **Competition Experiment**

North American and Chinese populations of *P. virginica* did not differ in above ground biomass when the interaction of competition was introduced ( $P = 0.217$ ; Table 7, Fig 7).

## CHAPTER IV

### DISCUSSION

#### Overview

*Plantago virginica* is a common herb residing along sidewalks and in the front lawns of many North American homes. Since its introduction into China 60 years ago, it has become a problematic immigrant in Chinese crop fields and amongst native biodiversity (Yan et al. 2000). The goal of this experiment was to examine if there were genetically based differences between North American and Chinese populations of *P. virginica*. Under the hypothesis that Chinese populations have evolved life history traits that have allowed the plant to progress into a more aggressive invader, a common garden study was conducted at Georgia Southern University. Results indicate that there are significant phenotypic differences in both growth and reproductive traits, suggesting that North American and Chinese populations of *P. virginica* have become genetically different since its introduction into China, which could contribute to its invasiveness in the new region.

#### Phenotypic Variation

The phenotypic variability within a region can differ between the native and invasive sites depending on the manner of its introduction. The calculated coefficient of variation used in my research is an effective estimator in indicating the extent of variability in relation to the mean of a population. By calculating this for observed traits and comparing the diversity in North American and Chinese populations from the common garden study, I was able to determine that Chinese populations had significantly less variability in phenotypic traits. Within the regions there were population level differences in the proportion of seeds that were able to germinate, the time it took inflorescences to emerge, and the number of infructescences on an individual. These

results could indicate that populations in North America exhibit more genetic variation than those in China, which could contribute to its ability to establish in a new area and produce offspring.

Both natural selection and genetic drift could alter genetic structures of populations in ways that modify their phenotypic variability. Together, these processes are the focus for the evolutionary mechanism of invasion (Lee 2002). The stochastic event of *P. virginica*'s invasion into China could be subject to natural selection because individuals not suited for the new area did not survive and produce offspring. Individuals that possessed advantageous traits for the new region were able to prosper and spread. This could contribute to the decrease in phenotypic variation found in this research. Similar research done by Blair and Wolfe (2004) showed that when comparing native European and invasive North American populations of *Silene latifolia*, selection in North America favored individuals in the invaded site that invested more energy into growth and reproduction and less into defense. They also determined that evolutionary forces like natural selection had an impact on the decreased phenotypic variability among populations. In these experiments with *P. virginica* and *S. latifolia* it is evident that selection has acted upon ecological forces that impacted its performance in the new range (Blair and Wolfe 2004, Bossdorf et al. 2004).

*P. virginica*'s introduction into China could have also been subject to genetic drift or more specifically, the founder effect. This is evident when levels of genetic variation in introduced species are often relatively low because of small founder population sizes (Wright 1929). In some cases genetic drift alone has promoted successful invasions (Lee 2002). For example Tsutsui et al. (1999) found that a population bottleneck caused reduced genetic diversity in invasive California ant populations leading to interspecific aggression in the invaded site. While the native Argentine populations exhibit intraspecific aggression, this bottleneck allowed invasive ants to eliminate native ant populations, attributing to its invasive success in California. In my research, Chinese populations of *P. virginica* could have begun from only a few members

of the original North American populations, which represent a portion of the phenotypic variation for the species, thus passing down a fraction of gene pool to the current Chinese generations. It is possible that seeds from more vigorous North American populations, by chance, were the source material in China, causing more aggressive North American genotypes to colonize and spread throughout the novel environment. These results demonstrate how simple population genetic changes can have dramatic ecological consequences.

Loss of phenotypic diversity can also be due to inbreeding depression. *P. virginica*, although wind pollinated, also has the capacity to self-fertilize. Invasive species with the ability to self-fertilize are a serious threat. According to Baker's Law (Stebbins 1957), species with selfing mechanisms are believed to be favored in population establishment (Barrett et al. 2008), because of the reproductive assurance. It is possible that, because of *P. virginica*'s ability to produce cleistogamous flowers, less phenotypic diversity in Chinese populations could be a consequence of inbreeding depression (Waller 1984). Walker et al. (2009) found similar results when comparing several invasive populations of *Impatiens glandulifera* in introduced European regions. They found decreased variation for several genes in all populations and attribute their results to small founder populations as well inbreeding depression.

Because this common garden experiment used field-collected seeds, we cannot rule out the impact maternal effects could have on the interpretation of our results (Roach et al. 1987). Large maternal effects have been demonstrated for seed size, which in turn, can affect several morphological and reproductive traits throughout the lifetime of a plant (Roach et al. 1987, Wolfe 1993). Although not collected in this experiment, the seed mass was collected in the Chinese common garden experiments that paralleled ours, and no difference was found when comparing regions. Also, genetic analysis revealed that North American populations had a greater number of alleles compared to their Chinese counterparts. Because of this and the comparable seed mass

between regions, results suggest that maternal effects did not influence other life history traits in *P. virginica*.

### **Morphological and Life History Traits**

I found significant overall regional differences in several germination, morphological and reproductive traits between the native North American and invasive Chinese populations of *P. virginica* in the greenhouse experiment. The identified differences in traits between regions could have occurred due to a combination of evolutionary forces previously described; but regardless of how they came about, these differences now being expressed in Chinese populations could be attributing to the aggressiveness of *P. virginica*'s invasion. This is consistent with the idea that plants in introduced ranges grow more vigorously than their native counterparts (Blossey and Notzold 1995).

Chinese populations had a considerable advantage in germination traits over North America populations. Because Chinese *P. virginica* had greater germination success and a tendency to germinate sooner, a larger number of offspring may be able to establish in an area before other native species. Also photosynthesizing sooner (as indicated by the earlier emergence of the first true leaf), Chinese populations may be producing their own energy making it more difficult for natives to out-compete them for space if their seeds have yet to establish. Chinese and North American populations also differed in leaf shape, but not photosynthetic area. Contributing to the aggressiveness of *P. virginica*'s invasion, the difference in leaf shape could be a result of decreased genetic variability due to an ecological force or this weedy species may have a universal performance advantage compared to native biodiversity (Dahler 2003).

Although populations of *P. virginica* from China possessed vigorous germination traits, North American plants were able to produce inflorescences and seeds in less time. North American populations may be making a trade off by allocating fewer resources to seed quality



and more to reproductive structure development. This approach of reproducing sooner may be beneficial in the native range, however, in order for *P. virginica* to be successful in China, individuals with a life history strategy of allocating resources to germination may be more successful in establishing and invading the new habitat. Chinese populations had longer, taller infructescences, but were unable to produce as many as North American populations. Because there was no difference in the total infructescence length per plant (average infructescence length\*number) and because infructescence length is a proxy of flower number (regression analysis) plants from both regions had the potential for the same reproductive output using different strategies. However, because of greater germination success, a plant from China has a greater likelihood of getting to the point of flowering and producing offspring compared to a plant from North America.

An intriguing result from the common garden experiment was that all North American and Chinese individuals produced cleistogamous flowers. The production of only cleistogamous flowers could be a major indicator of inbreeding depression in both Chinese and North American populations. Research in China that paralleled this experiment, found similar results in all four common gardens performed and experiments are currently being designed to explore this phenomenon (Dr. Hui Guo personal communication). This suggests that the production of open and closed flowers may be determined by environmental factors instead of genetics.

### **Effect of Competition Between Regions**

It has often been cited that competitive ability in an introduced species tends to contribute to its invasiveness in new regions (Baker 1991, Dahler 2003, Wolfe 2002, Wolfe & Blair 2009). However, I did not find significant differences in competitive ability between regions in the greenhouse experiment. In fact, plants in the presence of competition were able to have more above ground biomass than those in the absence of competition, regardless of the region.

When studying *P. virginica*'s competitive ability it is important to understand there are two aspects to competition. The first is the effect of the competitor on *P. virginica* and second the effect of *P. virginica* on the competitor (Goldberg et al. 1991) An important measurement that was not obtained in this experiment was the effect of *P. virginica* on the grass competitor. Populations of *P. virginica* were grown in the presence and absence of competition, but the competitor, *C. dactylon*, was not grown alone. In the event there were no regional differences in morphological traits of *P. virginica*, this measure would have shown if *P. virginica* was having a negative effect on the competitor.

These results from the competition experiment may be inconclusive because throughout the course of the experiment every individual was exposed to various temperature and water stressors. Approximately ten weeks after germination, plants were moved from 23°C to an unheated room with temperatures reaching 10°C at night. Also at this time, all plants were deprived of water for one week and then overwatered for the remainder of the experiment. It is important to note that every individual was exposed to the same stressor, allowing regional differences to still be assessed, however, the stress had a negative impact on plant growth, making it impossible to take most measurements. For example, some individuals never produced plant material after their first true leaf, while others sent up inflorescences too early, which were unable to form seeds. These results could have led to concluding that the means were not different when in reality they were (type II error), affecting the outcome of the study.

### Summary

After conducting this investigation into determining differences in phenotypic traits between North American and Chinese populations of *P. virginica*, results suggest that *P. virginica* has advanced to a more aggressive phenotype in the invaded range. Differences in genetic traits were unveiled by growing plants from the introduced and native ranges in a

common garden experiment as well as genetic data provided by our Chinese collaborators. Chinese plants have a decreased genetic variability and possess more vigorous traits compared to North American populations, which could be explained by natural selection, genetic drift (founder effects) or the combination of the two; uncovering the random chance that founding individuals possessed more aggressive North American phenotypes, survived in the new region because of these phenotypes, allowing this plant to spread successfully in the new region. This study is part of a collaboration with China in order to understand the mechanisms behind the invasion of *P. virginica*. The present study identified decreased phenotypic variation and more aggressive phenotypes in invasive populations. To further this research, an exploration into the mating system of *P. virginica* and what causes individuals to self-fertilize could provide insight as to how the plant has been able to colonize and if the selfing mechanism has allowed it to spread more rapidly in the new region.

Table 1: Identity and location of *Plantago virginica* populations.

N. America	ID	City	State	Latitude (N)	Longitude (W)	Elevation (m.a.s.l)
	1	Morehead City	N. Carolina	34°43'20.49"	76°45'30.22"	3
	2	Logan County	Oklahoma	35°43'53.48"	97°17'12.12"	329
	3	Edmond	Oklahoma	35°41'45.79"	97°20'44.74"	330
	4	Greensboro	N. Carolina	36°15'10.28"	79°45'24.74"	203
	5	Twin City	Georgia	32°33'48.92"	82°2'3.81"	71
	6	Statesboro	Georgia	32°25'13.54"	81°47'32.23"	66
	7	Statesboro	Georgia	32°24'8.27"	81°45'18.35"	33
	8	Gainesville	Florida	29°38'6.68"	82°20'20.03"	23
	9	Blackville	S. Carolina	33°19'57.89"	81°17'47.26"	57
	10	Palm Springs	California	33°49'22.80"	116°32'13.20"	106
China	ID	City	Province	Latitude (N)	Longitude (E)	Elevation (m.a.s.l)
	11	Nanjing	Jiangsu	32°03'06.93"	118°49'39.10"	50
	12	Nanjing	Jiangsu	32°02'24.97"	118°52'27.61"	74
	13	Jinhua	Zhejiang	29°08'08.76"	119°38'29.45"	74
	14	Nanchang	Jiangxi	28°39'11.02"	115°47'26.95"	18
	15	Nanchang	Jiangxi	28°41'17.22"	115°55'19.50"	21
	16	Nanchang	Jiangxi	28°41'14.39"	115°56'02.30"	24
	17	Fuzhou	Fujian	26°01'41.64"	119°12'25.48"	7
	18	Fuzhou	Fujian	26°03'39.60"	119°11'41.53"	11
	19	Shanghai	Shanghai	31°08'46.34"	121°26'11.68"	4
	20	Wuhan	Jianhan	30°32'38.66"	114°24'26.53"	35

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Table 2: Results for sources of variation in germination traits of *Plantago virginica*.

(\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 as indicated by F-values, df=1,18). Error reports as sum of squares.

<b>Trait</b>	<b>Region</b>	<b>Error</b>
Time to Germination (days)	3.25	14.23
Proportion of Seeds Germinated	6.76**	0.31

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Table 3: Results for sources of variation in morphological traits of *Plantago virginica*. (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 as indicated by F-values, df=1,17). Error reported as sum of squares, SLA=specific leaf area

<b>Trait</b>	<b>Region</b>	<b>Population (Region)</b>	<b>Error</b>
Time to First True Leaf (days)	1.69	2.08*	419.45
Plant size (# of leaves)	0.27	6.67***	1885.04
Leaf Length (mm)	0.98	3.46***	1827.19
Petiole Length (mm)	2.10**	4.12***	371.07
Leaf Width (mm)	2.17*	2.14**	234.35
Total Leaf Length (mm)	1.57**	4.37***	6092.59
Leaf Biomass (mg)	0.03	1.80*	0.10
SLA (mm <sup>2</sup> /mg)	0.0001	1.59	782000
Total Plant Area (mm <sup>2</sup> )	0.83	4.33***	3.19x10 <sup>7</sup>

Table 4: Results for sources of variation in reproductive traits of *Plantago virginica*. (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 as indicated by F-values, df=1,17). Error reported as sum of squares.

<b>Trait</b>	<b>Region</b>	<b>Population (Region)</b>	<b>Error</b>
Time to Inflorescence Production (days)	6.83***	7.97***	24226.49
Time to Seed Maturation (days)	10.97***	12.65***	42418.29
Number of Infructescences	5.75**	1.55	208.07
Peduncle Length (mm)	12.87***	12.95***	147018.11
Infructescence Length (mm)	6.41***	8.11***	29109.96
Total Infructescence Length Per Plant (mm)	0.032	0.93	100388.49

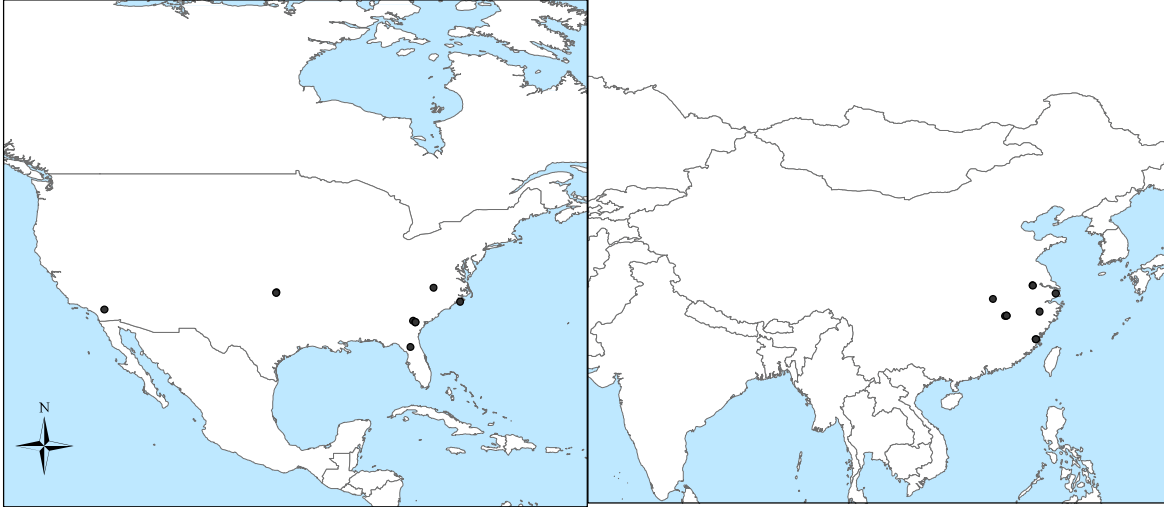




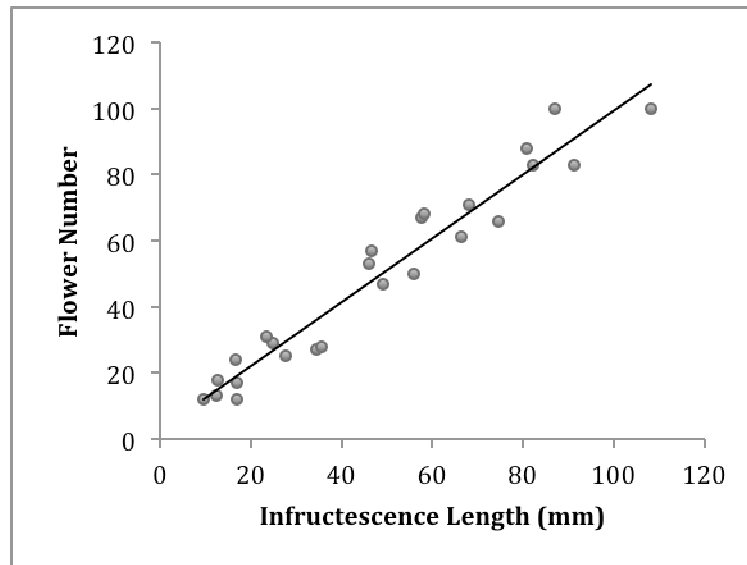


Table 7: Results for sources of variation in above ground biomass in *Plantago virginica* populations from North America and China in the presence and absence of competition (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 as indicated by F-values, df=1,19). Error reported as sum of squares

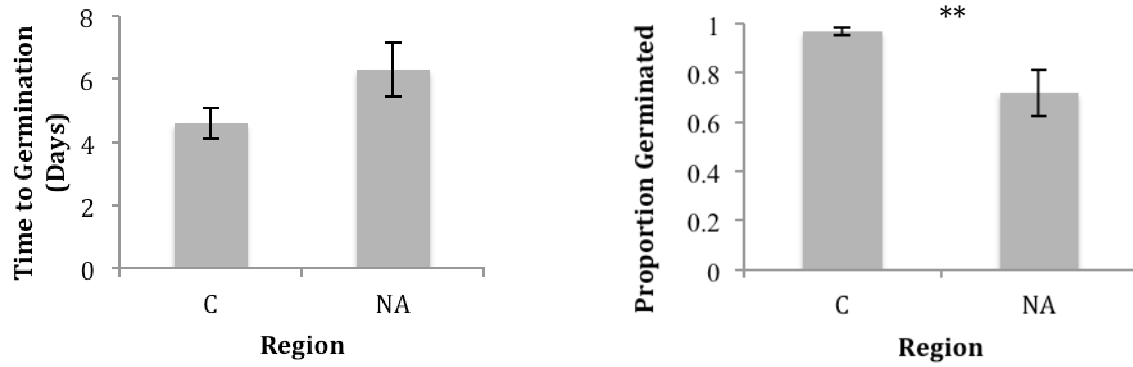
Region	Population (Region)	Competition	Competition*Region	Error
0.429	2.967***	0.0047*	0.216	0.014



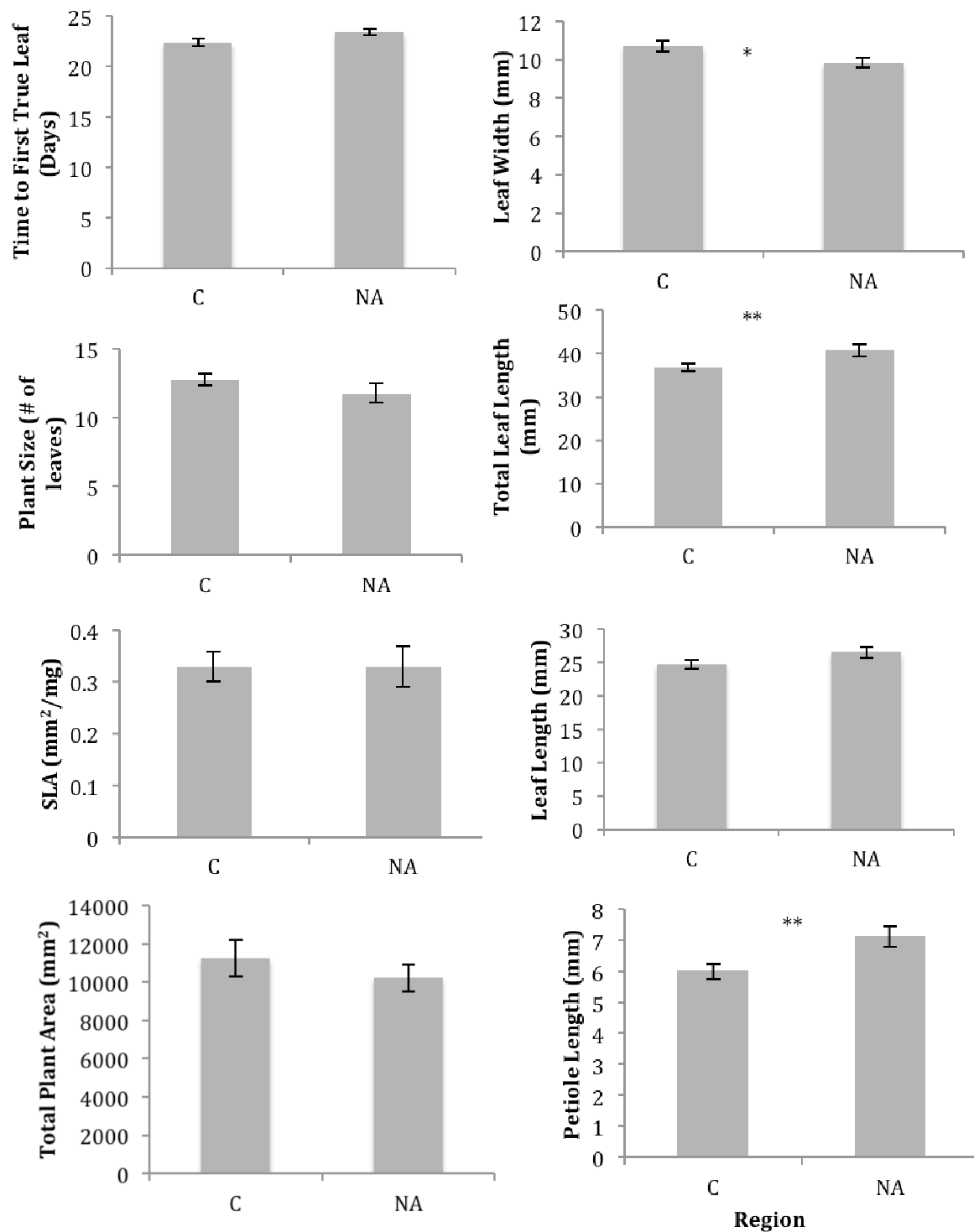
**Fig 1** Locations of collected *Plantago virginica* populations in North America and China. Projected coordinate system: GCS WGS 1984



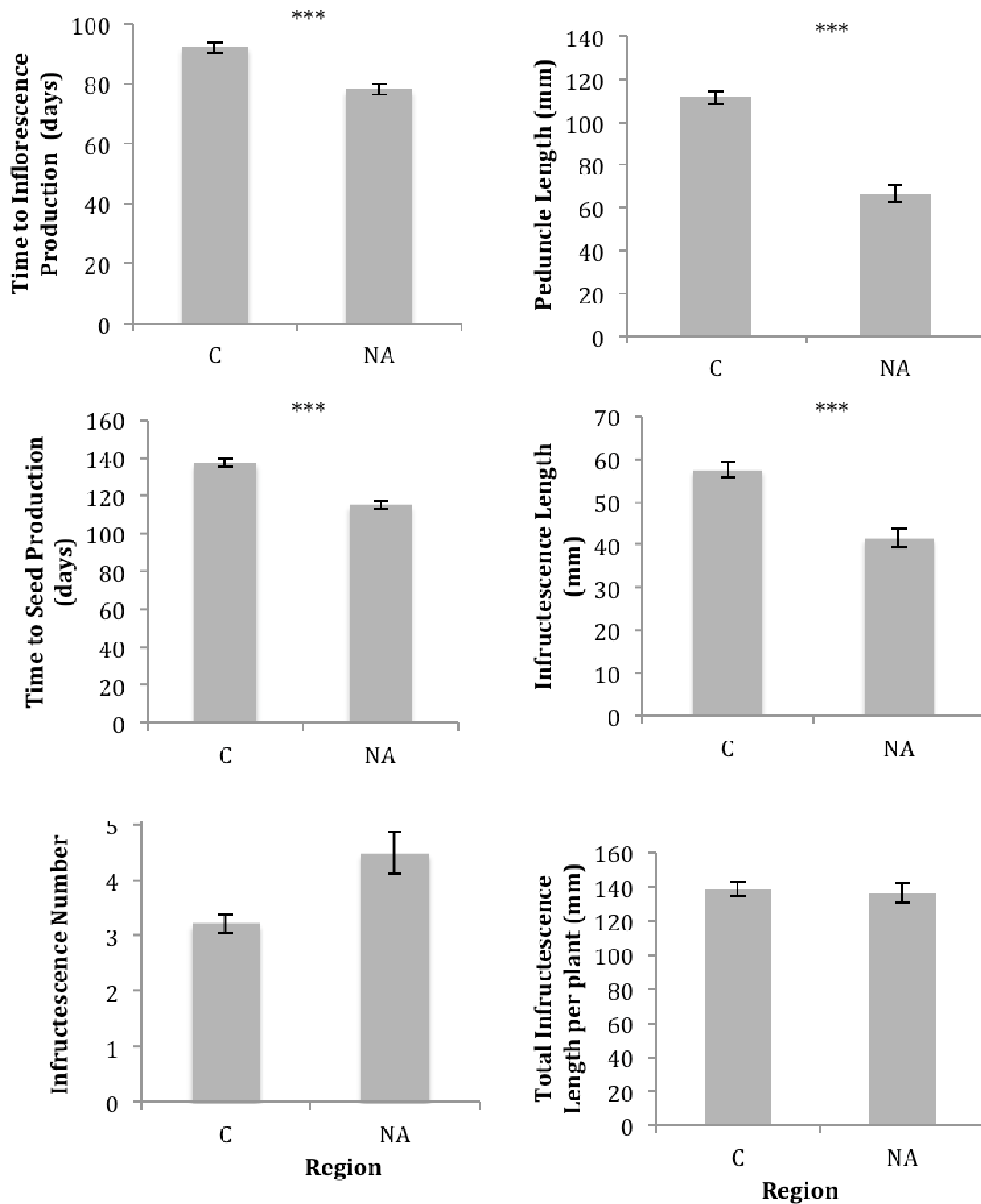
**Fig 2** Relationship between infructescence length and flower number in *Plantago virginica*



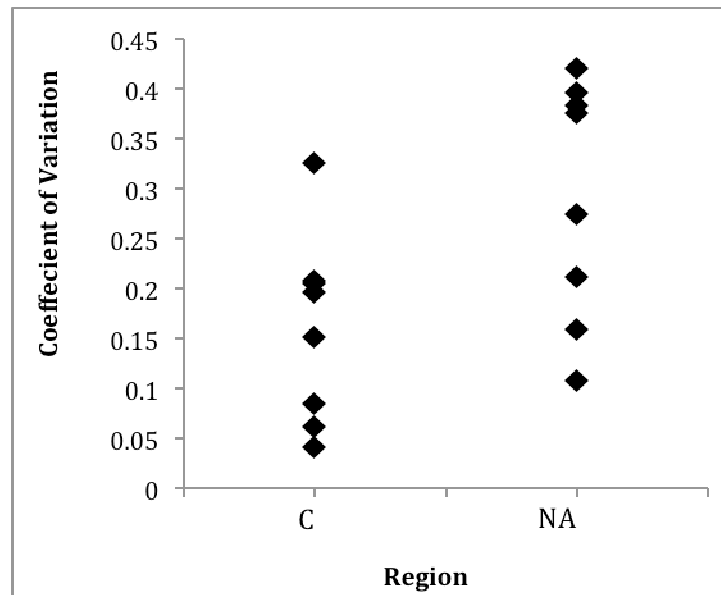
**Fig 3** Germination traits (mean  $\pm$  S.E.) of Chinese (C) and North American (NA) *Plantago virginica* populations in a common garden experiment (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). The values for the graph are found in Appendix C



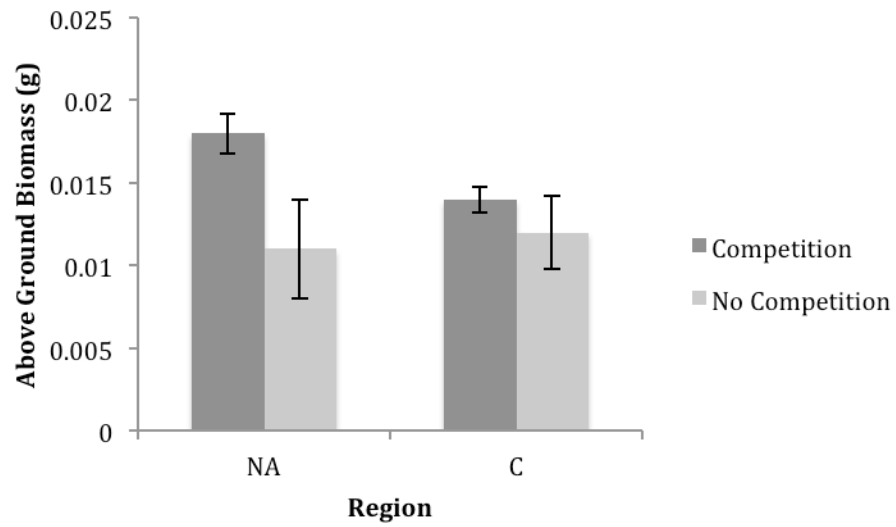
**Fig 4** Morphological traits (mean  $\pm$  S.E.) of Chinese (C) and North American (NA) *Plantago virginica* populations in a common garden experiment (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). SLA=Specific leaf area. The values for the graph are found in Appendix C



**Fig 5** Reproductive traits (mean ± S.E.) of Chinese (C) and North American (NA) *Plantago virginica* populations in a common garden experiment (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001). The values for the graph are found in Appendix C



**Fig 6** Coefficient of variation for selected traits of *Plantago virginica* from Chinese (C) and North American (NA) populations. The selected traits are as follows: time to germination (days), proportion of seeds germinated, size of plant (number of leaves), time to inflorescence emergence (days), infructescence length (mm), number of infructescences, total infructescence length per plant (mm), and time to seed maturation (days). Figures for particular traits are found in Appendix D



**Fig 7** Above ground biomass (mean $\pm$ SE) of *Plantago virginica* from North American (NA) and Chinese (C) populations in the presence and absence of competition. The values for the graph can be found in Appendix F.



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## **Appendices**

Appendix A: *Plantago virginica* with A: inflorescences that are producing seeds and B: Infructescences with mature seeds

A



B



Appendix B: *Plantago virginica* with A: chasmogamous (open) flowers (Gibson 2012) and B: cleistogamous (closed) flowers

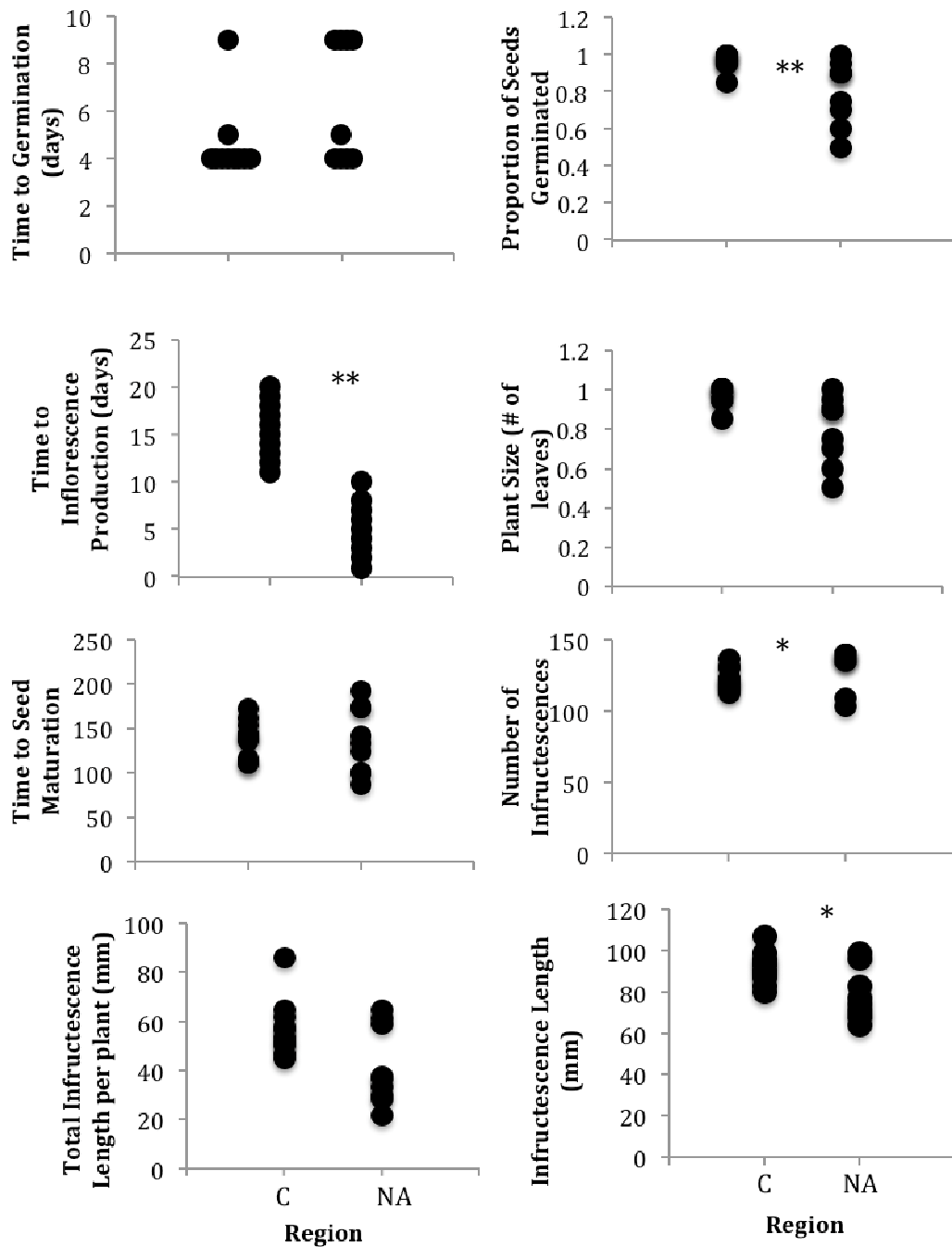


Appendix C: Descriptive statistics (mean  $\pm$  SE) of germination traits, vegetative traits, and reproductive traits for *Plantago virginica* in a common garden study at Georgia Southern University. SLA = specific leaf area

Trait	Region			
	China	North America	F	P
Time to Germination (days)	4.60 $\pm$ 0.49	6.33 $\pm$ 0.85	3.25	0.089
Proportion of Seeds Germinated	0.97 $\pm$ 0.02	0.72 $\pm$ 0.09	6.76	0.018
Time to First True Leaf (days)	22.36 $\pm$ 0.39	23.39 $\pm$ 0.34	1.70	0.062
Plant Size (number of leaves)	12.74 $\pm$ 0.43	11.76 $\pm$ 0.72	0.27	0.18
Leaf Length (mm)	24.75 $\pm$ 0.59	26.55 $\pm$ 0.81	0.98	0.067
Petiole Length (mm)	6.01 $\pm$ 0.24	7.14 $\pm$ 0.32	2.10	0.004
Leaf Width (mm)	10.72 $\pm$ 0.28	9.83 $\pm$ 0.28	2.17	0.033
Total Leaf length (mm)	36.78 $\pm$ 0.96	40.84 $\pm$ 1.34	1.57	0.010
Leaf Biomass (mg)	3.27 $\pm$ 0.20	3.19 $\pm$ 0.30	0.03	0.822
SLA (mm <sup>2</sup> /mg)	0.33 $\pm$ 0.03	0.33 $\pm$ 0.04	0.0001	0.99
Total Plant Area (mm <sup>2</sup> )	11245 $\pm$ 974	10209 $\pm$ 720	0.83	0.36
Time to Inflorescence Production (days)	92.20 $\pm$ 1.77	78.11 $\pm$ 1.73	6.83	<0.0001
Time to Seed Maturation (days)	137.43 $\pm$ 2.15	115.09 $\pm$ 2.08	10.97	<0.0001
Number of Infructescences	3.21 $\pm$ 0.16	4.48 $\pm$ 0.38	5.75	0.003
Peduncle Length (mm)	111.57 $\pm$ 2.97	66.39 $\pm$ 3.92	12.88	<0.0001
Infructescence Length (mm)	57.51 $\pm$ 1.78	41.49 $\pm$ 2.32	6.41	<0.0001
Total Infructescence Length per plant (mm)	138.78 $\pm$ 4.16	136.10 $\pm$ 5.70	34.69	0.86



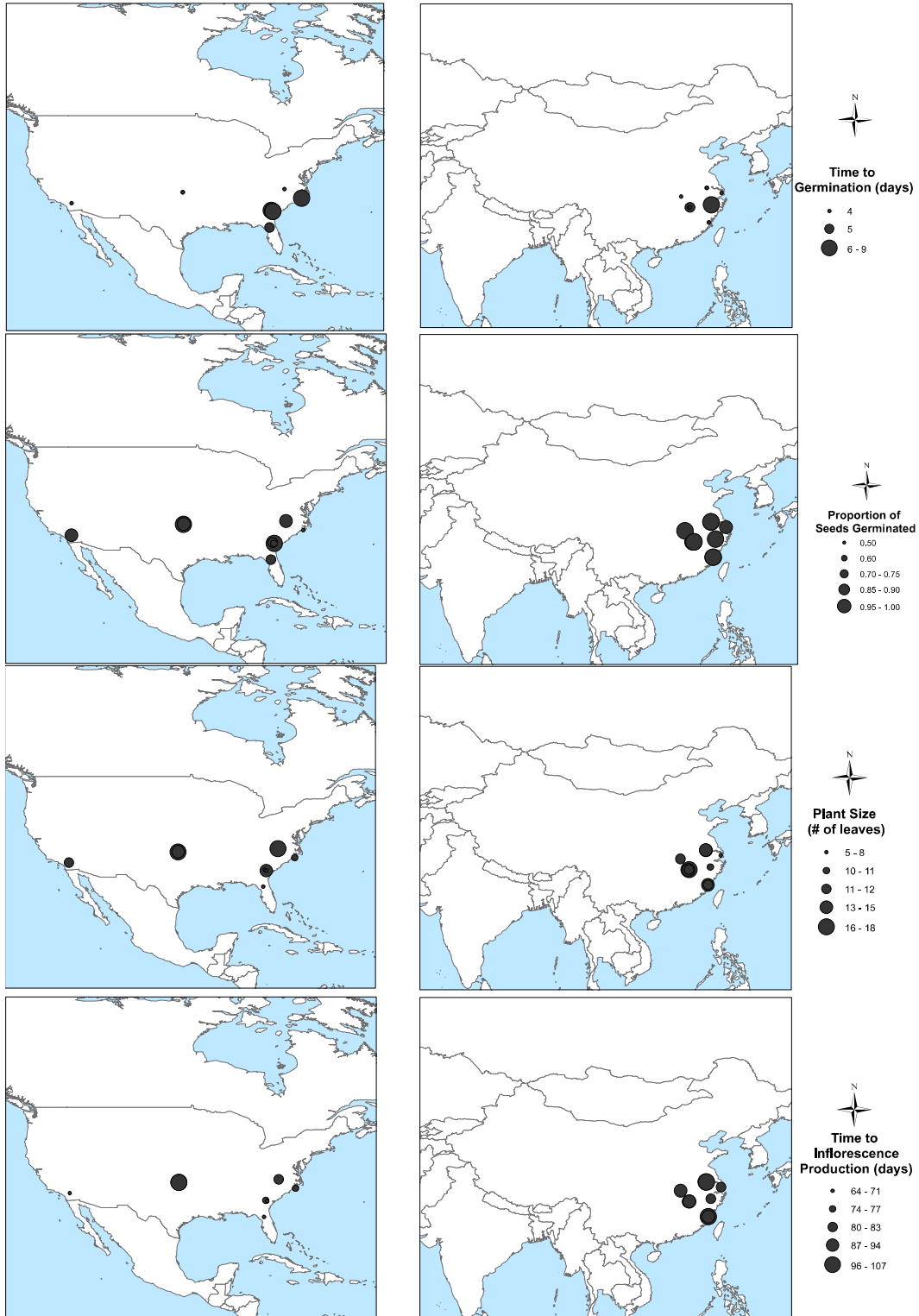
Appendix D: Population variation of selected traits in *Plantago virginica* populations from China (C) and North America (NA) presented as population means, coefficient of variation analysis is reported for each trait in Appendix E.



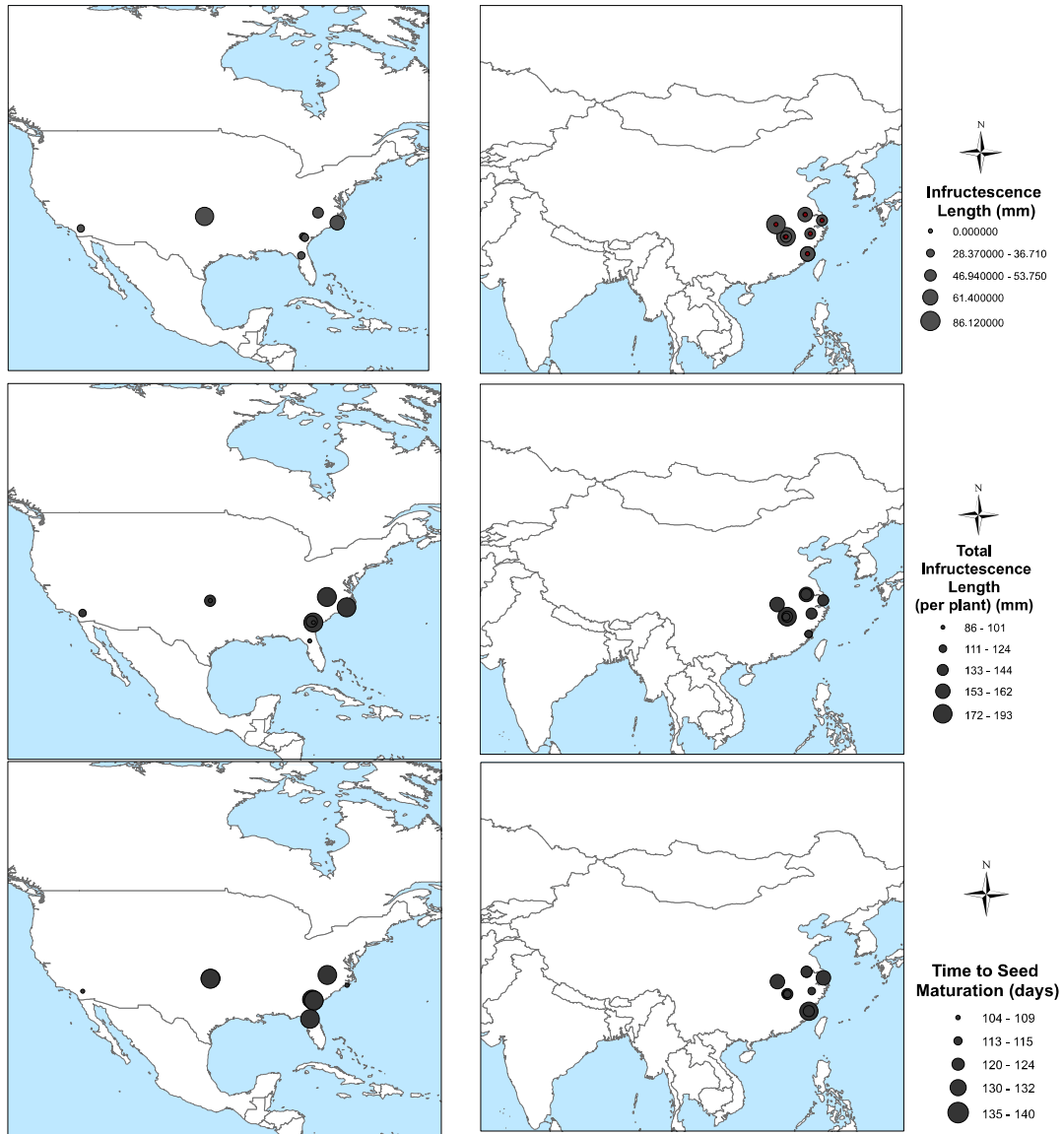
Appendix E: Coefficient of Variation (standard deviation/mean) for selected traits of *Plantago virginica* from the common garden study. (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 as indicated by t-value; df=1,18)

<b>Trait</b>	<b>North America</b>	<b>China</b>	<b>t</b>
Time to Germination (days)	0.39	0.32	3.25
Proportion of Seeds Germinated	0.21	0.04	9.09**
Size of Plant (# of leaves)	0.37	0.19	0.39
Time to Inflorescence Emergence (days)	0.16	0.09	8.70**
Infructescence Length	0.38	0.20	6.34*
Number of Infructescences	0.42	0.21	6.32*
Total Infructescence Length Per Plant (mm)	0.27	0.15	0.04
Time to Seed Maturation (days)	0.11	0.06	2.55

Appendix F: Locations for North American and Chinese populations of *Plantago virginica* represented as proportional symbols for selected traits. Projected coordinate system: GCS WGS 1984.



Appendix F: Continued



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Appendix G: Descriptive statistics (mean  $\pm$  SE) of above ground biomass for *Plantago virginica* in a common garden study at Georgia Southern University of competition between North American and Chinese populations. (F=1.54, P=0.217)

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	<u>North America</u>	<u>China</u>
<b>Competition</b>	0.018 $\pm$ 0.0012	0.014 $\pm$ 0.0008
<b>No Competition</b>	0.011 $\pm$ 0.003	0.012 $\pm$ 0.0022

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