EVOLUTIONARY AND DEMOGRAPHIC PROCESSES IN THE INVASIVE WEED *MICROSTEGIUM VIMINEUM*

by

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ABSTRACT OF THE DISSERTATION

Evolutionary and Demographic Processes in the

Invasive Weed *Microstegium vimineum*

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Microstegium vimineum (Trin.) A. Camus (stiltgrass) is considered among the most invasive plants in the eastern United States. There has been considerable study of this species' ecology and management though far less attention has been paid to its molecular ecology and the evolutionary processes which may influence its invasion success. Here, I describe a newly developed molecular marker system (microsatellite) which I used to examine *M. vimineum*'s genetic population structure and diversity in both its native and introduced ranges. I found clear signals that *M. vimineum*'s mating system is the most important determinant of the species' population structure and variability. The invasive range had lower genetic diversity overall, probably due to founder effects. Also, population and regional genetic differentiation appeared to be 'in process' in the invasive range. Furthermore, *M. vimineum*'s mixed cleistogamous/chasmogamous mating system allowed for the near fixation of microsatellite genotypes in a given population by high rates of selfing, while still

permitting the persistence of allelic diversity and generation of new genotypes at low frequency via occasional outcrossing. Thus, this mating system may confer adaptive advantage to the species as it settles upon fit genotypes in a given area while retaining evolutionary potential for range expansion into new habitats. I also attempted to discern adaptively significant phenotypes in *M. vimineum* through the measurement of phenological variation of plants originating from across the species' invasive range under manipulated light treatments. Flowering time and biomass were both strongly correlated with the latitude of population origin such that populations collected from more northern latitudes flowered significantly earlier at lower biomass than populations from southern locations. This pattern suggests rapid adaptive evolution of phenology over a period of less than one hundred years, and such changes have likely promoted the northward range expansion of this species. Interestingly, barriers to gene flow, including bottlenecks and inbreeding, have apparently not forestalled adaptive processes for this plant. Based on literature review and these new data, I hypothesize that adaptive evolution of phenological traits may be widespread in many invasive plant species and an essential process during range expansion.

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Chapter 1

An overview of the invasive grass *Microstegium vimineum*, with focus on distribution, physiology, ecology, management, and evolution

Abstract

A brief introduction to the distribution and physiology of *Microstegium vimineum* is followed by a review of ecological and evolutionary studies of the species. Special attention is given to relevant literature regarding phenological evolution and population genetics of the species. *Microstegium vimineum* is an invasive grass, native to eastern, southeastern and southern Asia. It has become a problematic invasive plant in disturbed habitats and forest understories in eastern North America, where it can outcompete native species and interfere with forest regeneration. To date, there has been extensive research into the ecology of *M. vimineum*, but little attention has been paid to relevant evolutionary processes that may be important to the species' invasion success. Even less attention has been given to molecular study of the species, with only one study examining population genetic structure of the species in a single watershed in Virginia.

Distribution and Physiology

Microstegium vimineum (Trin.) A. Camus is considered among the most invasive plants in the United States, with a wide distribution along the east and gulf coasts, as well as in the Midwest (USDA and NRCS 2008). It goes by several common names including, Japanese Stiltgrass, Stiltgrass, or Nepali Browntop. The species is an annual and native to Asia (China, Taiwan, Bhutan, India, Japan, Korea, Myanmar, Nepal, the Philippines, Russia, and Iran) (Chen and Phillips 2008), but has naturalized in North America and Turkey (USDA and NRCS 2008, Scholz and Byfield 2000; see Fig. 1.1). A member of the family Poaceae, subfamily Panicoiedeae, it is classified within the tribe Andropogoneae (Mathews et al. 2002). The genus *Microstegium* is characterized by paired spikelets, rambling culms, lanceolate leaf blades, sparsely hairy spikelets, with the lower glume concave to grooved along the median line. The species is distinguished from its congeners by the presence of a lower glume with transverse veinlets below the apex (Chen and Phillips 2008).

In North America, *M. vimineum* is sometimes confused with *Leersia virginica* during the vegetative growth phase, but it should be distinguishable by the presence of glabrous nodes and fibrous, non-rhizomatous, roots (Mehrhoff 2000). The first North American recording was in Knoxville, TN in 1919. By 1933, it was found in North Carolina and by 1972, was found from Florida to New Jersey, and westward to Ohio and Mississippi (Fairbrothers and Gray 1972). It is currently found and considered invasive as far north as Massachusetts (Mehrhoff 2000), as far west as Texas and Missouri, and as far south as Puerto Rico (see

Fig. 1.2). It is generally considered invasive in more than 20 U.S. states (USDA and NRCS 2005).

Microstegium vimineum is officially listed as a noxious weed in Alabama, Connecticut and Massachusetts, an invasive exotic in Tennessee (USDA, 2008), and is considered a serious threat to the integrity of natural areas in Illinois (Illinois Department of Energy and Natural Resources 1994). It has also been implicated in the alteration of forest fire regimes, with potential consequences for forest management (Luke Flory, personal communication).

Microstegium vimineum has two kinds of flowers that are produced in the late summer and early fall: chasmogamous flowers, borne on spikes that are terminal on the culm, and cleistogamous flowers, borne on spikes contained within the leaf sheaths of the upper two or three culm segments (Cheplick 2007; Chen and Phillips 2008; see Figs. 1.3-1.5). Chasmogamous flowers are capable of both self-pollination and cross-pollination from neighboring plants via wind since stigmas and anthers are exposed to the air at maturity. Cleistogamous flowers are enclosed by the leaf sheath in which they are contained and are thus fully self-pollinated due to the fact that the pollen is blocked from entering or leaving the flowers.

Although, *M. vimineum* exhibits a C_4 photosynthetic syndrome, it is nonetheless well adapted to the shaded conditions of the forest understory. Hortin and Neufeld (1998) found that *M. vimineum* possesses low dark respiration rates and low light compensation points, allowing maintenance of a

positive carbon balance during long periods of low light. They also found that when grown in high light, the plant was able to acclimate photosynthetically, while maintaining the shade tolerant attributes of low dark respiration rates, rapid stomatal movements in variable light, and low light compensation points. They hypothesized that its competitive superiority as an invader may stem from its ability to behave as a shade tolerant species, while maintaining the metabolism to increase carbon gains during sunflecks.

The species flowers during short days. Judge (2006) placed seeds from populations collected at three different latitudes of the invasive range into growth chambers, simulating short- (9 hour photoperiod) and long- (9 hour photoperiod with three hour light interruption of the dark period) daylight regimes. Regardless of temperature and growth stage, all plants flowered under short day conditions, while no plants flowered under long days, indicating that *M. vimineum* is an obligate short day plant. However, Bernier (1988) noted that flower production of short-day plants, in general, can also occur under long days, due to poor fertility, high irradiance, low temperature, root removal, or application of cytokinin. Though Judge (2006) did not examine the exact critical daylength period required to induce *M. vimineum* flowering, she noted that in North Carolina, the first inflorescences are visible in natural populations around the last week of September or the first week of October. This would suggest a critical photoperiod of around 12 hours, at least in North Carolina. Although Judge's three seed origins responded similarly to environmental cues for flowering, the fact that the experiments were run only at a 9 hour photoperiod may have masked ecotype

differences between populations. Judge (2006) further noted that differences among populations may become evident as day length approaches the critical flowering daylength.

Ecology, Competition and Evolution

Microstegium vimineum can colonize floodplains, streambanks, riparian slopes, roadsides, field margins, turf grass and other frequently disturbed habitats. Barden (1987) noted that in North Carolina, the plant was slow to invade undisturbed vegetation, but that it rapidly invaded disturbed, mesic, shaded floodplain areas such as scour prone locations and rights-of-way that are mowed. In Maryland and Washington D.C., Redman (1995) also found that *M. vimineum* invaded mesic and floodplain woodlands, and additionally listed shaded roadbanks, firetrails and logging roads as primary habitats. Secondary habitats included utility rights-of-way, thickets, and ditches. *Microstegium vimineum* is a successful competitor, capable of outcompeting native species in both disturbed and minimally disturbed habitats (Cole and Weltzin 2004; Belote and Weltzin 2006; Oswalt et al. 2007; Judge, Neal and Shear 2008), where it can then form dense monocultures (Barden 1987). Touchette and Romanello (2010) found that *M. vimineum*'s capacity to tolerate a range of soil moisture conditions, including the ability to maintain stable water relations during flooding and waterlogging, may facilitate the species' invasion of mesic habitats and disturbed systems.

There is evidence of a persistent seed bank. Barden (1987) determined that seeds remained viable for at least three years in a North Carolina floodplain. Gibson et al. (2002) noted that density of seedlings in the spring was greater than could be accounted for by the seed rain the preceding fall, indicating carryover from previous years, and noted that late season drought and other soil moisture considerations may influence seed production heavily. Seeds respond to cold stratification and, when stratified, germinate at a rate greater than 95% (Judge 2006), although there are anecdotal accounts that cold stratification is clearly not necessary to obtain germination rates greater than 90% (e.g., Luke Flory, personal communication; author's own observations). Schramm and Ehrenfeld (2010) found that understory shrub shade reduced both survival and seed set. They also found that seeds germinating above the litter layer experience higher mortality than those below, and hypothesized that the loss of shrub layer due to intense deer browse and other factors may accelerate the spread of *M. vimineum*.

Heubner (2010) observed colonization rates in a West Virginia forest. She found that most seeds did not move far from the mother plant but that plants were occasionally established up to 45 m from the maternal source. Since there was no clear pattern to the direction of this longer dispersal, she concluded that soil, water and animals are potential vectors. Average radial migration rates of stands were between 0.16 and 0.50 m per year. Forest interiors were estimated to be saturated with the plant in 10 to 59 years. The author posited that her results suggest accelerating spread rates in mesic forests, tempered by reduced

rates in drier and shadier areas, possibly as a result of decreased fitness in these environments.

Cheplick (2005) examined biomass partitioning and resource allocation by collecting seed families (i.e., seeds all collected from a single mother plant) from shady and sunny habitats in central New Jersey. For seeds germinated and grown in the greenhouse, tillers from shaded populations showed greater allocation to leaves but reduced allocation to seeds (from both cleistogamous and chasmogamous flowers), relative to plants from sunny populations, suggesting adaptive differentiation to light conditions in invasive habitats on a sub-population scale. Maternal family had significant effects on chasmogamous flower allocation and mean mass of all seeds. For mature plants harvested from the field, chasmogamous and cleistogamous allocations averaged 16% and 11%, respectively, in sunny habitat and 6% and 7% in shady habitat. There was no evidence of trade-off in allocation between the two flower types in greenhouse grown or in field collected plants, but after controlling for tiller size, the total mass of cleistogamous spikelets and seed production was significantly greater than chasmogamous production in plants from sunny habitats (0.0449 and 0.0199 for cleistogamous and chasmogamous allocation metrics, respectively). Gibson et al. (2002) found that in an old field succession site, dominated by secondary oak-hickory to early successional woody species, 62% of all seed production was cleistogamous. Cheplick (2005) concluded that *M. vimineum*'s ability to grow and allocate limited resources to seed production under deep shade conditions is crucial to the species' success as an invasive in disturbed forests, and noted that

the species may have arrived in its invasive range with this growth characteristic (i.e., preadapted). He, along with Gibson et al. (2002), also noted that chasmogamous reproduction, which allows outcrossing, occurred more in sunny habitats, whereas cleistogamous reproduction, which results in inbreeding, was favored under shaded conditions.

Cheplick (2006) further examined the modular aspects of plant growth on biomass allocation in *M. vimineum*. Working with populations from central New Jersey, he found that for vegetative and subterminal phytomers (i.e., whole plant modules [node to node in grasses], as opposed to comparisons between seeds or flowers), allocation was greatest to leaves and chasmogamous production in seed families from deep shade. For example, allocation to leaves was 31% in plants from shaded habitats vs. 26% in plants from sunny habitats, for vegetative phytomers. Allocation to chasmogamous spikelets was 18% in plants from shady habitats and 31% in plants from sunny habitats. Cleistogamous allocation decreased from terminal phytomers to subterminal phytomers, from 35% to 25% for plants from shady and sunny habitats, respectively. Both cleistogamous and chasmogamous seeds and flowers were positively correlated with leaf mass, suggesting that reproductive capacity is determined by available photosynthate. Cheplick (2006) concluded that a predominantly self-pollinating system, coupled with an annual life cycle, may be an especially favorable combination for *M. vimineum*. Moreover, the ability of the plant to adjust its modular allocation (including cleistogamous and chasmogamous inflorescences), in response to light conditions, via usage of distinct phytomers, maximizes its reproductive

fitness. Cheplick (2007) also found that *M. vimineum* biomass allocation to cleistogamous reproduction was over twice that of allocation to chasmogamous reproduction in edge habitats, but only 15% higher in shaded habitats, though the largest plants in the most resource-rich environments preferentially allocated more biomass to chasmogamy relative to cleistogamy, suggesting that chasmogamy is a plastically opportunistic mode of reproduction for this species.

To further examine the familial origin of growth trajectory and to determine whether reproduction mass scales with vegetative size, Cheplick (2008) planted seeds from 20 families (10 from each of two microsites: deep shade and sunny edge) in the greenhouse. Shoot dry mass was significantly related to microsite over time. Since the deviation in growth between microsite families took place primarily during the last two months of growth, Cheplick (2008) posited that late season growth increase enabled plants to maximize reproduction when light increased following canopy leaf senescence. Tiller number variation was significant at both the microsite and family levels (e.g., number of tillers averaged 12.08 and 13.78 for interior and edge microsites, respectively), potentially indicating both plastic and genetic control of this trait. Reproductive and vegetative mass per tiller were correlated for both microsites, suggesting that selection may favor larger tillers to increase seed output. In conclusion, Cheplick (2008) recommended both molecular and quantitative genetic investigations of variation within and among populations over a broad geographical area to provide a fuller picture of *M. vimineum* evolutionary processes in the invasive range.

Cheplick and Fox (2011) planted seedlings of *M. vimineum* at varying densities in greenhouse pots and exposed them to shaded and sunny conditions. Under shaded conditions, they found no density-dependent effects for reproduction, even though final shoot mass was significantly affected by both density and light treatments. Density yield curves for the sunny treatment revealed that solitary individuals could produce the same biomass as a group of competing individuals at higher densities. They suggested that *M. vimineum*'s success in woodlands may be due to a large range of density tolerances and an ability to set seed under shady conditions, even when densities are high. They further noted that the large size, with accompanying greater reproductive capacity, of plants in open, sunny areas (often found along roadsides and ditches) provided a major source of propagules able to colonize, following dispersal.

Ecosystem impacts of *M. vimineum* are numerous. For example, Oswalt et al. (2007) hypothesized that *M. vimineum* competes with regeneration of native woody plants. In a post-disturbance Tennessee forest understory, they determined that total native woody species stems per hectare declined with increasing *M. vimineum* cover ($p < 0.001$, $r^2 = 0.80$), as did simple species richness of native woody species ($p = 0.0023$, $r^2 = 0.47$). Ehrenfeld et al. (2001) found that *M. vimineum* invasion increased soil pH values and nitrogen mineralization rates in northern New Jersey. These effects on soils were consistent under natural (adjacent to the common understory species *Vaccinium pallidum*) and controlled (in a greenhouse in previously non-invaded soil)

conditions. Baiser et al. (2008) found that the species altered forest food webs in New Jersey forests during the period of 1980-2005, specifically via reduction of breeding woodland birds, due to the plant invasion's alteration of sub-canopy community structure. Interestingly, this food web effect resulted from an interaction with white-tailed deer (*Odocoileus virginianus*) after predator release led to deer overbrowsing and thus habitat creation for *M. vimineum*. Eschtruth and Battles (2009) also found evidence of deer accelerating *M. vimineum* invasion and Nuzzo et al. (2009) found evidence that exotic earthworms (of various genera) facilitate *M. vimineum* invasion. Simao et al. (2010) recorded arthropod decreases of 39% in abundance and 19% in species richness from experimentally introduced *M. vimineum* plots. Finally, Baurer and Flory (2011) found that *M. vimineum* suppressed the native herb *Senna hebecarpa*, but found no evidence that the suppression effect was mediated by plant-soil interactions, thereby implicating direct competition effects, as opposed to indirect effects on soil nutrition via alteration of soil microbial communities, as contributing to *M. vimineum*'s success in this case.

In order to study the effect of light availability on competition in *M. vimineum*, Flory et al. (2007) planted pots with 95% *M. vimineum* and 5% *Dichanthelium clandestinum* seeds under a range of natural canopy shade levels in Indiana. They found that even with the unequal initial seed mix, *D. clandestinum* dominated under high light conditions, while *M. vimineum* dominated under low light conditions. In addition, they also planted their *Microstegium/Dichanthelium* seed mixture in pots with tillers of native graminoids.

They found that the invasion treatment decreased overall biomass of the resident community under partial shade treatment but not under full sun or full shade treatments.

Flory and Clay (2010) established 32 experimental plots in a bottomland, semi-shaded, hardwood forest field site where they planted with 12 native species, and then added *M. vimineum* seed, in an effort to determine the direct impact of invasion on native communities. These plots were monitored for species composition for two years and biomass for three years. Invasion reduced native biomass by 46, 64 and 58%, respectively, over three growing seasons, but resulted in higher total community biomass in two out of three years. After the second year of invasion, plots had 43% lower species richness and 38% lower Shannon diversity. Native species did not gain competitive dominance after multiple growing seasons, even though their plots were open to recruitment of many nearby species. They also found that native plants were more strongly suppressed in densely invaded areas.

A leading hypothesis to explain species invasions suggests that invasive species evolve following their introduction. The Evolution of Increased Competitive Ability (EICA) hypothesis posits that invasiveness of non-indigenous plants is a result of shifts in biomass allocation patterns. In the absence of herbivores, selection favors genotypes with improved competitive abilities and reduced resource allocation to herbivore defense (Blossey and Notzold 1995). In other words, since these species leave their herbivorous enemies behind and no longer need to defend themselves, they can rapidly evolve greater competitive

traits such as faster growth rates utilizing metabolic resources no longer needed for defense. Flory et al. (2011a) showed that plants from the invasive range of *M. vimineum* grew larger under common garden conditions than those from China. They found that introduced populations had higher biomass, despite lower allocation to leaves, suggesting greater photosynthetic efficiency. They concluded that their results are consistent with the EICA hypothesis. However, it should be noted that no one is entirely sure how many times *M. vimineum* entered North America or from precisely whence. It remains entirely possible that differences observed between plants from the invasive and native ranges may reflect phenotypic variation already extant within the native range, rather than having evolved in North America, post introduction.

Recognizing that studies conducted under a limited set of environmental conditions may show inconsistent results if native and introduced populations are differentially adapted to specific conditions, Flory et al. (2011b) studied origin x environment interactions by planting seedlings from 10 native and 10 invasive *M. vimineum* populations in 22 common garden experiments in Indiana. The common garden plots were specifically chosen to represent a range of habitats, including mowed fields, shaded bottomland forests, dry forested ridge tops, stream banks, and forest edges. On average, North American *M. vimineum* produced 46% greater biomass and had 7.4% higher survival than Asian plants. There was no evidence of greater plasticity based on seed origin.

Droste et al. (2010) exposed seven invasive *M. vimineum* populations to drought stress in a growth chamber and then chose the two most divergent

populations for growth in the greenhouse, under both drought and shade manipulation. *Microstegium vimineum* showed plasticity for biomass production and specific leaf area, and populations varied significantly in the degree of plasticity under both treatments, which they suggested could be an evolved trait in the invasive range. They concluded that *M. vimineum* either did not experience a genetic bottle-neck during invasion, that repeated introductions have negated any previous bottleneck, or that there has been rapid evolution since introduction. It should be noted, however, that these experiments were all conducted on plants grown from seed, as opposed to some sort of clonal propagation, raising the possibility that some of the recorded population plasticity could have resulted from varying degrees of genetic diversity within each sampled population.

Evolutionary Biology, Phenology and Invasion

Evolutionary processes can be fundamental to the process of invasion (Novak 2007). The genetic composition of recently established populations of an invasive colonizing species can provide important insights into the mode of population establishment (Pappert et al. 2000), as well as contributing to our understanding of rapid evolutionary processes (Lee 2002). In addition to the theoretical value of understanding how and why biological invasions occur, the design and success of control strategies, especially for potential biological control agents depends on knowing the origin, character, and geographical extent of genetic diversity within and among invasive populations (Valiant et al. 2007).

Invasions of species, following their introduction into new ranges may be due to biotic or abiotic characteristics of invaded habitats, traits of the introduced species, or some combination of both (Catford et al. 2009, Gurevitch et al. 2011). Theoretical and empirical studies suggest that evolution of introduced populations may be an underappreciated aspect of biological invasions (Baker 1974, Lee 2002, Novak 2007, Lankau et al. 2009, Dormontt et al. 2011). Introductions of species may result in founder effects, genetic drift, novel hybridization events, or adaption to novel environments (Bossdorf et al. 2005), and post-introduction evolution may explain the lag time before many species become invasive (Crooks 2005). Specifically, rapid evolution has been noted as an important process during both range expansion and invasion (Maron et al. 2004, Montague et al. 2007, Xu et al. 2010), since the introduction of a species into a new range often involves exposure to new selective regimes (Suarez and Tsutsui 2008). Genetic changes in introduced populations may allow invaders to adapt to novel environments, gain a competitive advantage over resident species, and undergo rapid range expansion (Blossey and Notzold 1995, Maron et al. 2004, Xu et al. 2010, Buswell et al. 2011).

Agriculturalists have long been artificially selecting (consciously or unconsciously) plant varieties with appropriate phenology (i.e., the seasonal timing of reproduction and other life history events) for their environment in order to expand the range of specific agronomic species. On page 121 of his 1898 fictional work *Etidorhpa*, John Uri Lloyd noted the apparent trade off between size at reproduction and appropriate latitudinal phenology for corn (*Zea mays*):

> ...Indian corn in Kentucky is luxuriant, tall, and graceful, and each stalk is supplied with roots to the second and third joint, while in the northland it scarcely reaches to the shoulder of a man, and, in order to escape the early northern frost, arrives at maturity before the more southern variety begins to tassel.

In natural systems, phenology has been shown to be responsive to various selective pressures (Griffith and Watson 2006, Franks et al. 2007). In particular, genetically controlled phenological timing has been associated with fitness benefits through interaction with frost avoidance (Kuser and Ching 1980), climate change (Bradshaw and Holzapfel 2001), growth rates (Blair and Wolf 2004), defense responses (Meyer and Hull-Sanders 2008), reproductive rates (Brown and Eckert 2005), plasticity (Lavergne and Molofsky 2007), and trade-offs with size at reproduction (Colautti et al. 2010).

Management Strategies

Microstegium vimineum has frequently been ranked as an invasive species whose control is a priority, but control has (to date) been difficult (e.g. Drake et al., 2003). Hand weeding, mechanical, chemical and cultural practices are all possible control methods. Hand weeding, mowing and weed-whacking are recommended in late summer or early fall before seed set. Flooding for at least three months or intermittently during the growing season may be an effective control (Tu 2000). No biological control agents for the species are yet reported, but a newly discovered fungal pathogen in the genus *Bipolaris* may hold promise (Kleczewski and Flory 2010).

Judge et al. (2005a) evaluated a suite of pre- and post-emergence herbicides, already registered for large crabgrass (*Digitaria sanguinalis*), for control of *M. vimineum*. They found that most pre-emergence herbicides used to control large crabgrass in turf and landscapes also control *M. vimineum*, though Benefin plus oryzalin, dithiopyr, isoxaben plus trifluralin, trifluralin, oryzalin, oxadiazon, pendimethalin, or prodiamine were the best performers, with control of 87% or greater, when compared with no treatment. They also found that post-emergence applications of clethodim, fenoxaprop-P, fluazifop-P, or sethoxydim resulted in 50 to 88% control. For broad spectrum herbicides, they found that two applications of glufosinate or one application of glyphosate provided control.

In order to evaluate herbicide treatments under more realistic field conditions and include effects on the ecological impacts of long-term management strategies for the species, Judge et al. (2005b) compared mechanical treatments, herbicide treatments, and a combined treatment over three growing seasons in an invaded forest in North Carolina. While all treatments significantly reduced *M. vimineum* cover, when compared with no treatment, recruitment of native plants was highest in the combined treatment of hand-removal and fenoxaprop-P. In addition, relative cover of other invasive species decreased across all treatments, with the exception of season long hand-removal, which increased relative cover of other invasives by 51%.

Flory (2010) evaluated hand weeding, a post-emergent graminoid specific herbicide (fluazifop-P-butyl), and post-emergent herbicide plus pre-emergent herbicide (pendimethalin) in southern Indiana. He found that natural systems

invaded by *M. vimineum* are best restored utilizing the post-emergent treatment or hand weeding over multiple seasons, though multiple hand weedings over a season were not advised, due to prohibitive labor costs. Post-emergent herbicide alone was also an effective control treatment and promoted recovery of native communities. Pre-emergent herbicide treatments removed *M. vimineum* but inhibited recovery of native communities. Local light conditions did not alter the effectiveness of treatment.

In reviewing the available literature on *M. vimineum*, Warren II et al. (2011) examined stage-specific weaknesses in the plant's life history to glean potential management strategies. They noted the importance of understory *M. vimineum* population as sinks fed by inputs from populations in higher light; they echo the call to eradicate *M. vimineum* sources made by others (e.g., Huebner 2010). They further note that the species may be dispersal-limited and reliant on anthropogenic transport, a character that, if confirmed, could present an effective management opportunity. Finally, they suggest that greater nitrogen deposition could be leveraged to increase the competitive ability of native species and that increased drought could create conditions unfavorable to the species.

Population Genetics

To date, there has been very little work done on any genetic aspect of *M. vimineum*. A search of GenBank for the species revealed only five nucleotide sequences deposited, the typical nuclear and chloroplast genes and spacers

used in plant systematics, used to place the genus *Microstegium* within the tribe Andropogoneae of the Poaceae. The only published example of a landscape level population genetics study of the species is an AFLP (Amplified Fragment Length Polymorphism) study conducted in a single watershed (James River Basin) in Virginia (Baker and Dyer 2011). The authors genotyped 359 individuals from 23 populations with AFLPs and found evidence for three separate introductions into the watershed and a zone of secondary contact between two of the distinct lineages discovered. Mean diversity, as measured by Shannon's *I*, was 0.264. AMOVA (Analysis of Molecular Variance) yielded significant differentiation among populations, both within regions (defined as a central, east and west sections; $\Phi_{SR} = 0.17$, $p < 0.005$) and among all populations ($\Phi_{ST} = 0.55$, $p < 0.005$). They found a heterogeneous distribution of diversity among populations and, contrary to initial expectations, no evidence of continuous expansion in a westward direction. They concluded that in their study region, there is evidence of long distance dispersal, with no obvious direction of spread, and diffuse gene flow over relatively short distances, with connectivity among populations. They suggested that management strategies should therefore focus on both preventing long distance dispersal and eradication of newly established populations.

Examination of genetic structure in other species with the mixed cleistogamous/chasmogamous mating system may provide important insights into what patters of allelic variation one should expect in *M. vimineum*. Other species with similar mating systems exhibit patterns of genetic variation within

populations that are typical of inbreeding species, but inter-population divergence that is more similar to out-crossing species. For example, in *Impatiens capensis* (Jewelweed), also an annual plant with mixed cleistogamous/chasmogamous reproduction, mean within-population heterozygosity per individual was found to be low, and population structure was found to be compatible with Wright's Island model (Knight and Waller 1987). The evolution of the mixed cleistogamous/ chasmogamous system has been related to cost-benefit analyses of flower production (Schoen 1984) and variation in fertility of seeds produced by the two floral types (Masuda et al. 2001), but the role of selfing vs. non-selfing systems as a determinant of allelic frequency change and fixation, which has been discussed by others (e.g., Allard and Workman 1963), should also be carefully examined in species such as *M. vimineum*.

Conclusions

Microstegium vimineum is an invasive grass native to eastern, southeastern and southern Asia. It has become a problematic invasive plant in disturbed habitats and forest understories in eastern North America, where it can outcompete desirable species and interfere with forest regeneration. To date, there has been extensive research into the ecology, physiology, management and distribution of *M. vimineum* in North America. Few studies have compared the species in its invasive and native ranges, and little attention has been paid to relevant evolutionary processes that may be important in the species' success in

North America. Even less attention has been given to genetic study of the species, with a single study that examines population genetic structure in a single watershed in Virginia as the only example.

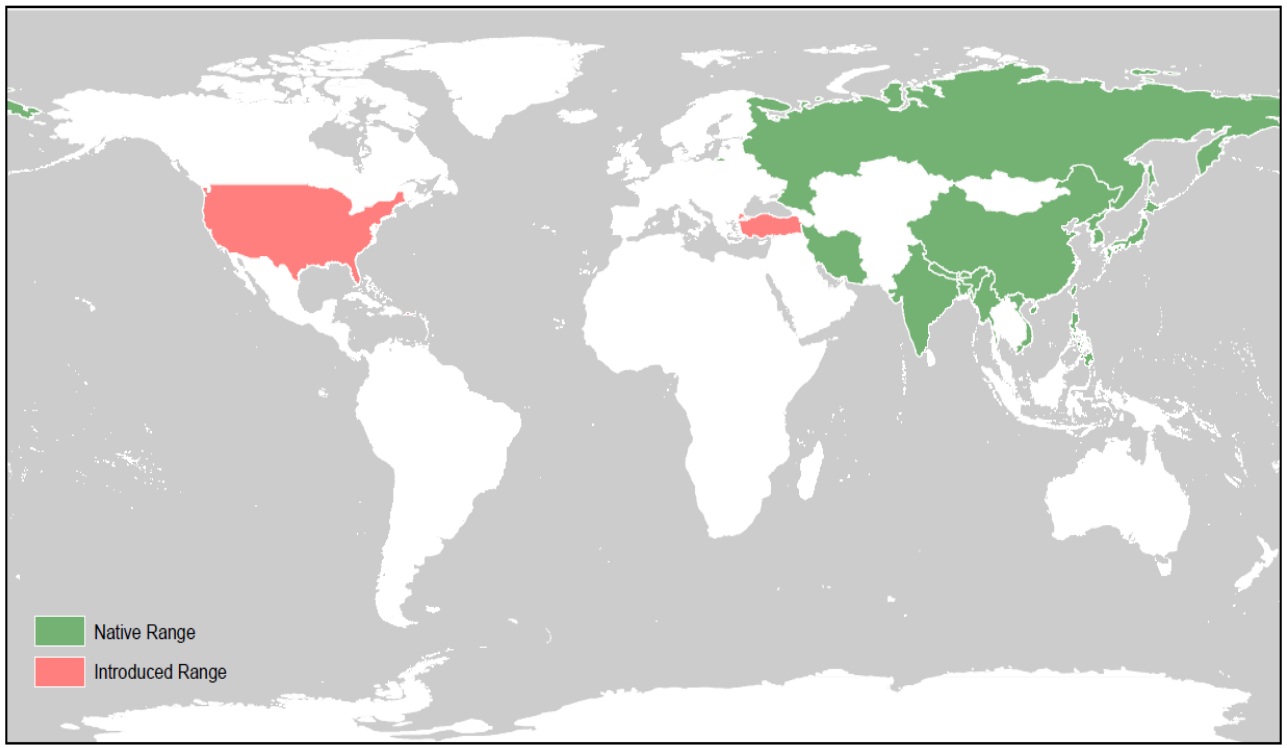


Figure 1.1 Global distribution of *M. vimineum* by country. Green indicates countries in which *M. vimineum* is considered native. Red indicates countries where the species is considered introduced or invasive. Note: In most countries with *M. vimineum*, the species is not present in the entire country.

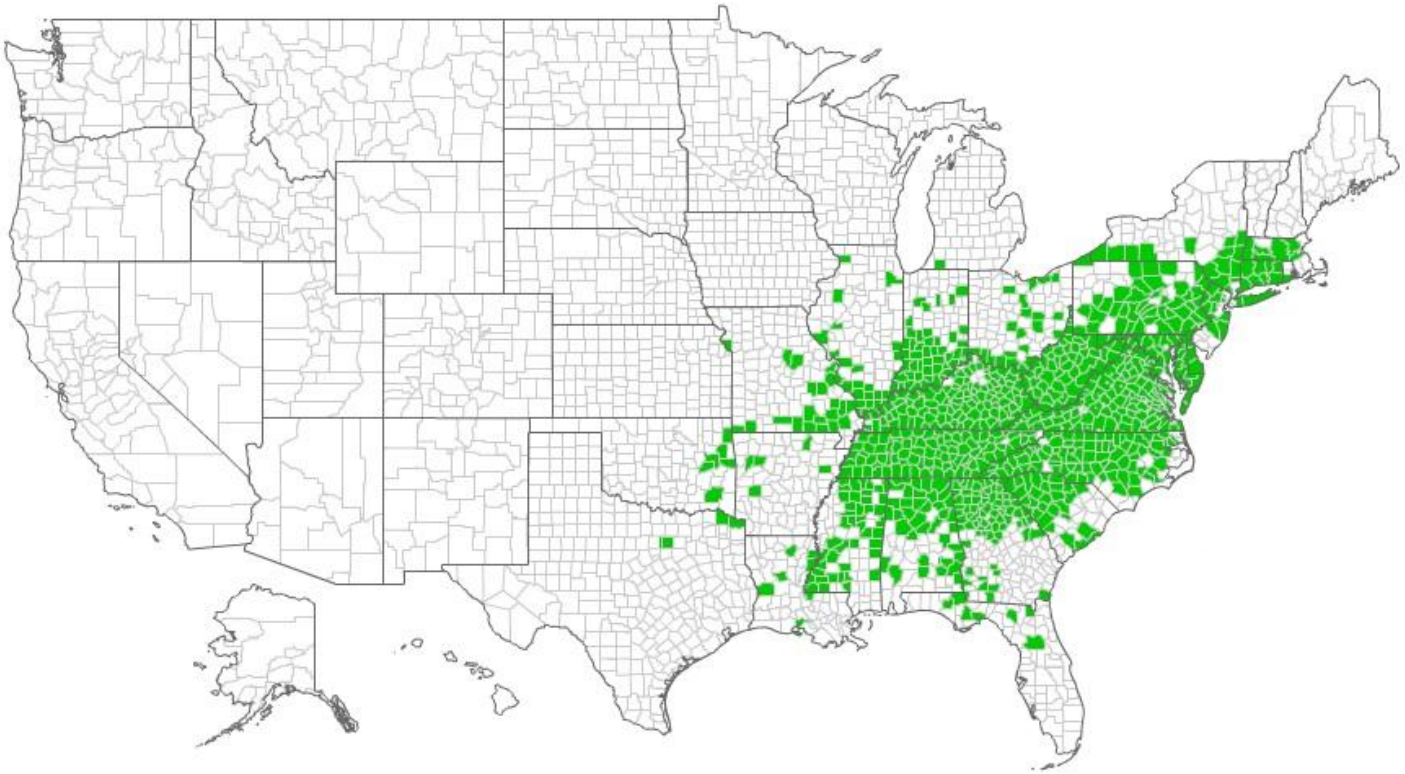


Figure 1.2. Approximate distribution and range extents of *M. vimineum* in the United States. Map adapted from www.eddmaps.org (Early Detection & Distribution Mapping System, University of Georgia). Note: *M. vimineum* is also naturalized in Puerto Rico.

Figure 1.3. Dense stand of *M. vimineum* in a New Jersey woodland.

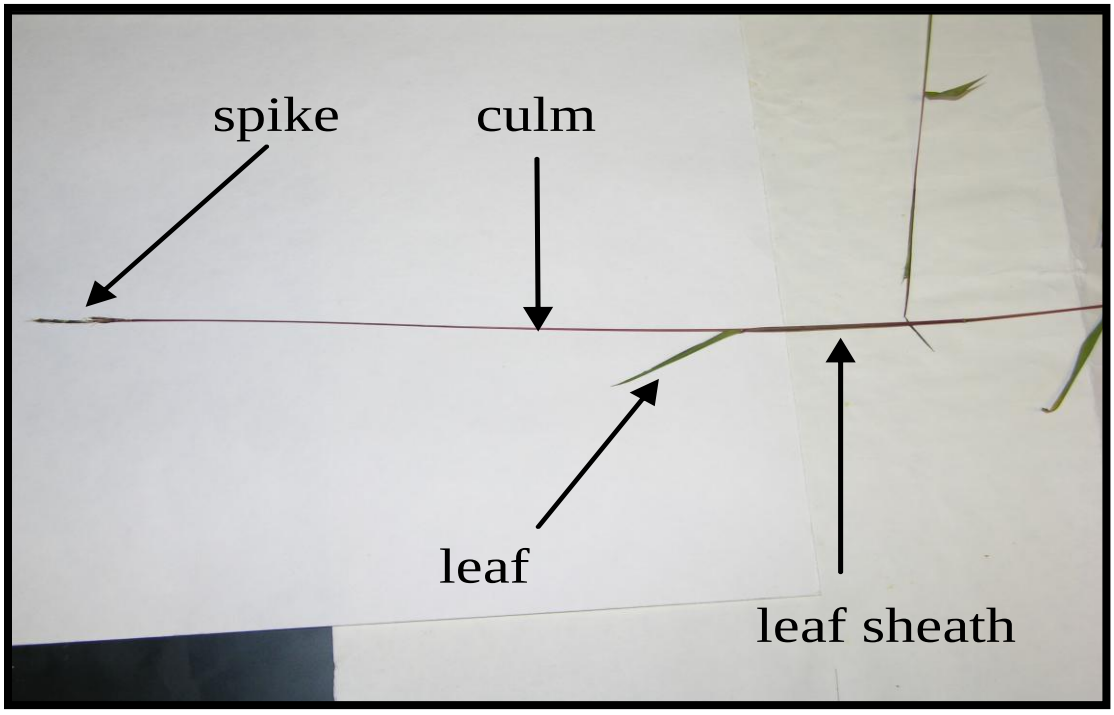


Figure 1.4. Terminal, chasmogamous spike of *M. vimineum*, which is accessible to out-crossing via wind pollination. Note: *M. vimineum* is usually an upright species. This picture was taken on a horizontal table. The terminal spike would normally be the highest, vertical element of the plant.

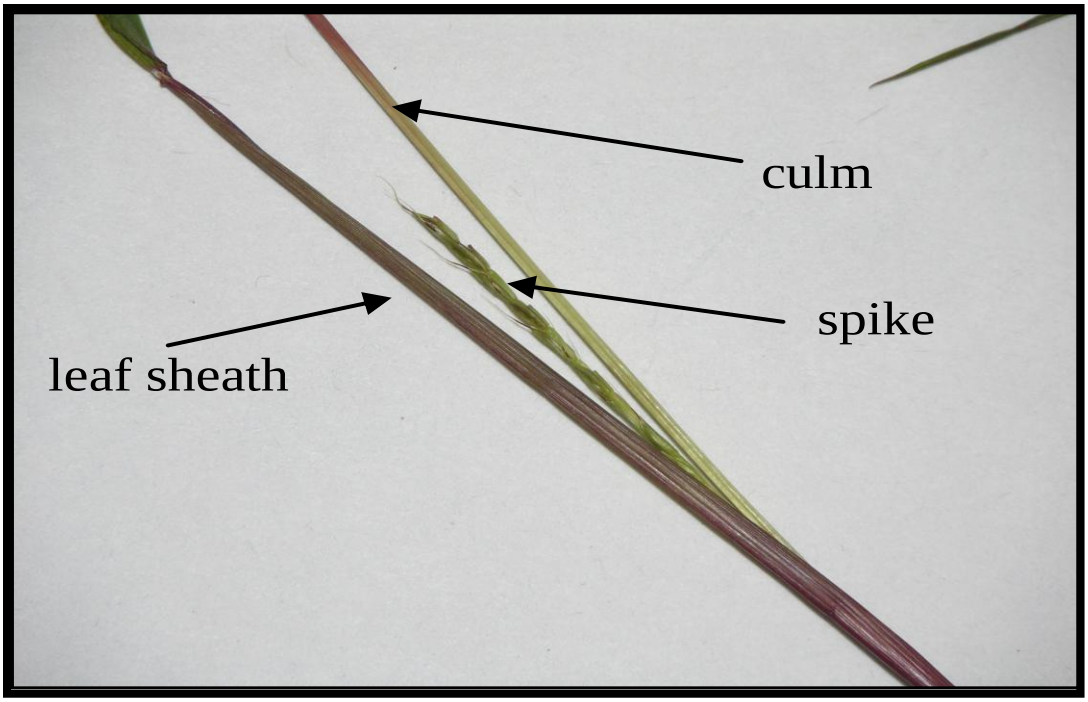


Figure 1.5. Cleistogamous spike of *M. vimineum* seeds revealed when the leaf sheath is pulled away from the stem. At pollination the cleistogamous flowers are wholly contained within the leaf sheath**.**

Chapter 2

Characterization of polymorphic microsatellite loci in *Microstegium vimineum*

Abstract

Microsatellite markers were developed for the invasive plant *Microstegium vimineum* (Poaceae) to assess its population genetic structure and to facilitate tracking of invasion expansion. Using 454 sequencing, 11 polymorphic and 6 monomorphic microsatellite primer sets were developed for *M. vimineum*. The primer sets were tested on individuals sampled from six populations in the United States and China. The polymorphic primers amplified di-, tri-, and tetra-nucleotide repeats with three to ten alleles per locus. These markers will be useful for a variety of applications including tracking of invasion dynamics and population genetics studies.

Note: A modified version of this chapter has been accepted for publication by the *American Journal of Botany*.

Introduction

Microstegium vimineum (Trin.) A. Camus, also known as Japanese stiltgrass, is considered among the most invasive plants in the eastern United States. It is a diploid, C4 annual grass native to much of eastern Asia, including China, Taiwan, Bhutan, India, Japan, Korea, Myanmar, Nepal, the Philippines, Russia, and Iran (Chen and Phillips 2008). A member of the grass family (Poaceae), classified within the tribe Andropogoneae and the subfamily Panicoideae (Mathews et al. 2002), it has invaded habitats throughout much of eastern North America and Turkey (Scholz and Byfield 2000). *Microstegium vimineum* produces two types of flowers: cleistogamous flowers borne on spikes, contained within the leaf sheaths, and chasmogamous flowers borne on the terminal spike (Cheplick 2007, Chen and Phillips 2008). *Microstegium vimineum* was first recorded in North America in Knoxville, TN, in 1919 but may have been introduced elsewhere. It was documented in North Carolina in 1933, and was found from Florida to New Jersey, and west to Ohio and Mississippi, by 1972 (Fairbrothers and Gray 1972). *Microstegium vimineum* is currently found and considered invasive as far north as Massachusetts (Mehrhoff 2000), as far west as Texas and Missouri, and as far south as Puerto Rico (USDA and NRCS 2008). Here I report 17 nuclear microsatellite loci for *M. vimineum* developed using 454 next-generation sequencing.

These markers were developed to facilitate studies into population genetics and structure of the species in both the native and invasive ranges. In chapter 3 I will describe in detail the justification and aims associated with the

invasive and native range population genetic analyses that these markers make possible. In brief, this marker system is expected to allow for calculation of standard population genetics parameters including allelic frequency, heterozygosity, other measures of genetic diversity, genetic distance, fixation index, and genetic structure (via AMOVA,PCO and other methods). These data will then be examined to determine biologically relevant parameters including the appropriate spatial scale for defining populations and regions in this species. I will also compare various metrics between the native and invaded range to determine evolutionary processes which may be affecting *M. vimineum* invasion. I will be paying particular attention to signs of bottlenecks (reduction in genetic diversity in the invasive range) and other limitations of gene flow, as these characteristics often indicate important information about adaptational potential of a species during range expansion. This process of generating a basic understanding of the overall genetic structure of the species is fundamental to subsequent studies described in this thesis. In addition to laying the groundwork for an evolutionary understanding of the invasion process, it provides the basic information necessary for tracking invasion dynamics.

Materials and Methods

I initially planned to use the 384 conserved intron scanning primers (CISPs) developed for orphan species within the Poaceae (Feltus et al. 2006) to identify polymorphic markers within populations of *M. vimineum*. After testing 120

CISPs against *M. vimineum* samples from throughout its invasive range, I found 6 markers which amplified consistently and well in *M. vimineum*, but none which were polymorphic. As such, that avenue did not seem adequate for obtaining enough markers, with enough variation, to adequately describe *M. vimineum* population genetics. As a result, I applied for and received funds, with Dr. J.M. Hartman, through the USDA McIntire-Stennis program at the Rutgers New Jersey Agricultural Experiment Station, to leverage 454 sequencing to discover novel microsatellite markers for the species. Ultimately, the 454 sequencing strategy proved successful.

One *M. vimineum* sample from New Brunswick, NJ, USA (40.4760° N, 74.4241° W) was sequenced by 454 pyro-sequencing at the Savannah River Ecology Laboratory (Aiken, SC, USA). The 454 sequencing technique is described in detail in Abdelkrim et al. (2009) and Lance et al. (2010) and followed the enrichment procedure of Glenn and Schable (2005). Briefly, DNA was digested with restriction enzyme *Rsa*I (New England Biolabs, Ipswich, MA, USA), ligated to double-stranded linkers, denatured and hybridized to biotinylated microsatellite oligonucleotide mixes (mix 2 = $(AG)_{12}$, $(TG)_{12}$, $(AAC)_6$, $(AAG)_8$, $(AAT)_{12}$, $(ACT)_{12}$, $(ATC)_8$; mix 3 = $(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACAG)_6$, $(ACCT)_6$, $(ACTC)_6$, $(ACTG)_6$; mix 4 = $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(AGAT)_8$), then captured on magnetic streptavidin beads (Dynal, Invitrogen Coroporation, Carlsbad, CA, USA). Unhybridized DNA was washed away and remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the SimpleX-10 as a primer. Barcoding to

distinguish *M. vimineum* samples from other samples pooled in the 454 run was accomplished using the custom linkers SimpleXL10_U (5'-AAAGCAGCGTCGGAATG -3') and SimpleXL10_Lp (5'-pCATTCCGACGCTGC -3'). The enriched libraries were sequenced on a Roche 454 pyro-sequencer using titanium chemistry following standard Roche 454 library protocols (454 Life Sciences, a Roche company, Brandford, CT, USA). Sequences were subjected to a 3' quality trim where only one base in the last 25 bases of the sequence contains a quality score less than 20 or alternatively contains one ambiguous base. CAP3 (Huang and Madan 1999) was then used to assemble sequences at 98% sequence identity using a minimal overlap of 75 bp. Sequence data were screened using MSATCOMMANDER 0.8.2 (Faircloth 2008), which also allows for primer design using PRIMER 3 (Rozen and Skaletsky 2000). A total of 475 putative primer pairs were designed, including 60 tetranucleotides, 143 trinucleotides, and 272 dinucleotides.

I chose 81 of these putative primer pairs (20 tetra-, 32 tri-, and 29 dinucleotides), based on the calculated lowest potential of primer interaction, and amplified them against eight *M. vimineum* samples from throughout the species' invasive range. Of these, 4 tetra-, 14 tri-, and 4 dinucleotide primers amplified well. These 22 primer pairs were amplified against 95 samples from the United States and China. One sample from each population used in this study has been vouchered at the Chrysler Herbarium (CHRB; accession numbers: Novy 2-7), Rutgers University (New Brunswick, NJ, USA). I amplified template DNA by PCR, according to the protocol described by Schuelke (2000). Conditions of the PCR

amplification were an initial heating of 94°C (5 min), followed by 30 cycles of 94°C (30 s)/56°C (45 s)/72°C (45 s), then 20 cycles of 94°C (30 s)/53°C (45 s)/72°C (45 s), and a final extension at 72°C for 10 min. Each PCR reaction included the attachment of a FAM, NED, PET, or VIC fluorescent label. I genotyped PCR products on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA), using a LIZ 500 size standard, and identified and binned alleles using GeneMapper 3.7 software (Applied Biosystems, Foster City, CA, USA). Resulting genotypic data was analyzed in GenAlEx ver. 6 (Peakall and Smouse 2006) to calculate observed (H_O) and expected (H_E) heterozygosity for each polymorphic locus over each population and over all populations. All primer sequences have been submitted to the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/).

Results

Table 2.1 lists the 11 polymorphic and 6 monomorphic loci which amplified well in *M. vimineum*. I include the monomorphic loci here since they may potentially be useful in future studies of *M. vimineum* and other related species. Five of the 22 primer pairs mentioned above were discarded since they appeared to amplify multiple genomic regions and/or were otherwise difficult to score. For all loci, I report the primer name, sequences of the forward and reverse primers, microsatellite motif and repeat number in the sequenced individual, size range of fragments across all samples, theoretical primer melting temperature determined

by PRIMER 3 (Rozen and Skaletsky 2000), whether or not the locus is polymorphic, and the GenBank accession number (Table 2.1). For all samples in all populations, the number of alleles per polymorphic locus ranged from 3 to 10. The values for number of alleles (N_a), observed (H_O) and expected (H_E) heterozygosity for each polymorphic locus over each population are given in Table 2.2. Each of these values is also averaged for all loci to present a basic measure of the marker systems' overall descriptive power.

Discussion

I have characterized 17 microsatellite loci for the invasive grass *M. vimineum*. The 11 polymorphic loci all behaved as we would expect in a diploid (i.e., exhibiting one or two alleles per sample); however, I did record a high proportion of homozygosity, probably as a result of the species' tendency to inbreed. Though these results are gathered from a relatively limited set of populations, this is most likely an indicator that populations exhibit relatively low allelic diversity. Similarly to the high homozygosity noted, this may be a consequence of high rates of inbreeding as rarer alleles are more quickly purged from populations and a small number of dominant 'fixed' genotypes take over. Though care should be taken when analyzing a small dataset primarily meant to validate a set of markers, a few patterns do present themselves here. Most interestingly, there does appear to be higher allelic diversity in Asian populations when compared with invasive North American populations. N_a ranged from 1.00-

2.09 in North American populations and from 1.36-2.36 in Asian populations despite the fact that more plants were sampled in North American populations. This may be a first indication that bottlenecks due to patterns of introduction during invasion have decreased genetic diversity in the invasive range. This is further evidenced by the higher observed heterozygosity in Asian (0.06-0.22) vs. North American (0.00-0.10) populations. Finally, the lower rates of observed heterozygosity, as compared to expected heterozygosity, is another indicator of how high levels of inbreeding maybe shaping population genetic structure in this species. In summary, these markers appear to contain ample diversity, and potentially divergent structure, to be useful for a variety of applications including tracking of invasion dynamics and further population genetics studies.

Primer	Sequence	Repeat	Size	T_a (°C)	Polymorphic	GenBank
MV01	F: CCAGTGAATGTCATTTGTCC R: GCGTGAATTGAAATGATTG	$(AG)_{10}$	227-250	60	Yes	JN247840
MV02	F: CTCTGCAGCTATCGATCAAC R: GATGGCCCATAGAACTAGTG	$(AG)_9$	224-228	61	Yes	JN247841
MV03	F: GTCTGACCACCAACATTCTG R: TTCAGGAAAGCTACCCTATG	$(AAG)_{16}$	309-358	60	Yes	JN247842
MV04	F: CAAATGTCCTTGTCCTCATC R: GGTTGGGTATATTTGGAATG	$(ATC)_7$	387-400	60	Yes	JN247843
MV05	F: CATGCCAACCCTATTCTATC R: GAGAAACAAGGTGCAAAGAG	$(AAC)_7$	383-428	60	Yes	JN247844
MV06	F: AGCATCTTTACCGGTATGAC R: ATGTCCAACGAACAAAGAAC	$(AAG)_7(AGG)_{11}$	303-347	60	Yes	JN247845
MV07	F: CCTCCTTCAGACAGTCATTG R: TACAACAGATGCCGACTACC	$(AAC)_8$	367-378	61	Yes	JN247846
MV08	F: AATGACAAGTGATCGAGTGG R: TCCATCTCGTCGTGTAATAAC	$(ACT)_{10}$	305-324	60	Yes	JN247847
MV09	F: TCATCCATCTCCATAACTCC R: TTGCCATCTTCCCTACTAAC	$(ACAT)_{11}$	117-137	60	Yes	JN247848
MV10	F: TGAAGACAATGAGGCAAGTC R: TCGTCCTTGTGAGTCATGAC	$(AAAC)_6$	262-283	60	Yes	JN247849
MV11	F: ATGGTGTTCGATGAAATGTC R: TAACCATTCCAACCAATTTC	$(AGAT)_7$	296-336	61	Yes	JN247850
MV12	F: AAATGATAAGCCCGTTTAAG R: ACACCACGACTAAAGACAGC	$(AGAT)_6$	131	60	No	JN415631
MV13	F: TCCCATGAAACTTGACAGAG R: TGAAGTATTCGGCTCTGAAG	$(AAG)_{11}$	246	61	No	JN415632
MV14	F: ACCAGACCAGGCTAGAGATC R: TTCGGTCAACAAGTCACC	$(ATC)_7$	437	61	No	JN415633
MV15	F: TTTCTTCACTCCACCTTCTG R: GTCAACCAAGAGCAGAACC	$(AAG)_{21}$	189	60	No	JN415634
MV16	F: AGGTTACATTGCACCCATAC R: CTCGATCGTCTTCAGCTTAC	$(AC)_{11}$	259	60	No	JN415635
MV17	F: TTAGGTGACCCAACAACATC R: GATTGCTCCAAACTCTAAGC	$(AC)_8$	365	60	No	JN415636

Table 2.1. Characteristics of 11 polymorphic and 6 monomorphic microsatellite primers developed in *Microstegium vimineum*. Shown for each primer pair are the forward and reverse sequence, repeat motif, size range of the fragments (bp), annealing temperature (T_a), and the GenBank accession number.

	China 1 (N=11) Zhe Jiang Province 30.1748° N, 119.1990° E			China 2 (N = 13) Zhe Jiang Province 30.2567° N, 119.7228° E		
Locus	**Na**	H_O	H_E	**Na**	H_O	H_E
MV01	3	0.09	0.37	2	0.08	0.08
MV02	3	0.00	0.43	1	0.00	0.00
MV03	3	0.09	0.37	1	0.00	0.00
MV04	1	0.00	0.00	3	0.25	0.45
MV05	2	0.18	0.17	1	0.00	0.00
MV06	3	0.09	0.37	1	0.00	0.00
MV07	2	0.00	0.44	1	0.00	0.00
MV08	2	0.00	0.44	2	0.08	0.07
MV09	3	0.09	0.37	1	0.00	0.00
MV10	2	0.09	0.35	1	0.00	0.00
MV11	2	0.00	0.30	1	0.00	0.00
Average	2.36	0.06	0.33	1.36	0.04	0.06

	China 3 (N= 10) Shanghai Province 31.3593° N, 121.3593° E			USA 1 (N=18) New Jersey 40.5886° N, 74.5630° W		
Locus	**Na**	H_O	H_E	**Na**	H_O	H_E
MV01	2	0.11	0.40	1	0.00	0.00
MV02	2	0.10	0.50	2	0.00	0.49
MV03	2	0.30	0.38	2	0.00	0.49
MV04	3	0.33	0.44	3	0.28	0.37
MV05	2	0.00	0.18	3	0.00	0.54
MV06	3	0.33	0.43	2	0.00	0.49
MV07	1	0.00	0.00	2	0.00	0.50
MV08	2	0.38	0.43	3	0.00	0.55
MV09	4	0.33	0.44	2	0.00	0.49
MV10	2	0.11	0.10	1	0.00	0.00
MV11	3	0.44	0.43	2	0.00	0.49
Average	2.36	0.22	0.34	2.09	0.03	0.40

Table 2.2. Results of initial primer screening for 95 samples of *Microstegium vimineum* from 6 populations in China and the United States for 11 polymorphic microsatellite loci. For each locus, the number of alleles (Na), observed heterozygosity (H_o), and expected heterozygosity (H_E) are reported.

	USA 2 (N=21) New York 41.3084° N, 74.0003° W			USA 3 (N=22) South Carolina 34.0491° N, 81.1828° W			All samples (N=95)
Locus	**Na**	H_O	H_E	**Na**	H_O	H_E	**Na**
MV01	1	0.00	0.00	1	0.00	0.00	6
MV02	1	0.00	0.00	1	0.00	0.00	3
MV03	1	0.00	0.00	1	0.00	0.00	6
MV04	1	0.00	0.00	2	0.05	0.04	5
MV05	1	0.00	0.00	1	0.00	0.00	4
MV06	1	0.00	0.00	3	0.00	0.18	10
MV07	1	0.00	0.00	1	0.00	0.00	4
MV08	1	0.00	0.00	1	0.00	0.00	5
MV09	1	0.00	0.00	1	0.00	0.00	7
MV10	1	0.00	0.00	1	0.00	0.00	3
MV11	1	0.00	0.00	2	1.00	0.50	5
Average	1.00	0.00	0.00	1.36	0.10	0.07	5.27

Table 2.2 (Cont.). Results of initial primer screening for 95 samples of *Microstegium vimineum* from 6 populations in China and the United States for 11 polymorphic microsatellite loci. For each locus, the number of alleles (Na), observed heterozygosity (H_o), and expected heterozygosity (H_E) are reported.

Chapter 3

Population genetic analysis *of Microstegium vimineum* in its native and introduced ranges

Abstract

On a fundamental level, it is important to understand the post-colonization invasion path of a rapidly spreading species such as M*icrostegium vimineum.* Its genetic structure, the level and pattern of variation within and among populations and regions, represents a persistent signature of the colonization process. For this study, I assayed 34 populations of *M. vimineum*, 10 from the native range and 24 from the invasive range. I found clear indications that the mating system of *M. vimineum* is the most important determinant of the continental and sub-regional level population structure observed. *Microstegium vimineum*'s mixed cleistogamous/chasmogamous mating system yields near fixation of genotypes within any given population, while still preserving additional genetic diversity at low frequency. This system may confer adaptive advantage for the species, as it settles upon different optimal genotypes in different areas, while retaining evolutionary potential for range expansion. The invasive range exhibited less genetic diversity than is present in the original range, probably due to founder effects. Also, population and regional genetic differentiation appeared to be 'in process' in the invasive range, as further divergence and differentiation are likely to continue as the species further expands and settles into its invasive range.

Introduction

Microstegium vimineum (Trin.) A. Camus is a C4 annual grass native to Asia, where it is found in various habitats, including forest margins and riparian areas (Chen & Phillips 2007). Its first North American herbarium record was filed in 1919 in Tennessee; it had expanded to North Carolina by 1933; and was found from Florida to New Jersey, and west to Ohio and Mississippi, by 1972 (Fairbrothers and Gray 1972). Though initially noticed in disturbed areas such as riparian and road corridors, the plant has subsequently become established in mature forests (Barden 1987, Oswalt et al. 2007). It is currently found and considered invasive as far west as Texas and Missouri, as far south as Puerto Rico (USDA, 2008) and as far north as Massachusetts, with range expansion continuing (Mehrhoff 2000).

On a fundamental level, it is important to understand the mode of colonization of a rapidly spreading invasive species such as *M. vimineum.* Its genetic structure, the level and pattern of variation within and among populations and regions, represents a persistent signature of the colonization process (Pappert et al. 2000). A careful analysis of that genetic structure can be expected to increase our understanding of the demographic determinants and, possibly, the evolutionary trajectories of such rapid expansion.

In addition to the theoretical value of understanding how and why biological invasions occur, the design and success of control strategies, especially potential biological control agents, depends on knowing the origin,

character, and geographical extent of genetic diversity within and among invasive populations (Valliant et al. 2007). The basic population biology of an organism, as revealed by its population genetic structure, may suggest vulnerable life history stages or other targets that may be amenable to managerial intervention, developing control practices and predicting invasion potential (Allendorf and Lundquist 2003).

Microstegium vimineum has two kinds of flowers: cleistogamous flowers (Fig. 1.5) borne on spikes contained within the leaf sheaths of the upper two or three culm segments, and a chasmogamous flowers (Fig. 1.4), terminal on the culm (Cheplick 2007, Chen and Phillips 2008). Cleistogamous flowers are self-pollinated, as pollen is blocked from entering or leaving the flowers by the leaf sheath that contains them. Chasmogamous flowers are exposed to the air and are capable of both self-pollination and cross-pollination from neighboring plants via wind. The evolution of this mixed cleistogamous/chasmogamous system has been related to cost-benefit analyses of floral production (Schoen 1984) and variation in fertility of seeds produced by the two floral types (Masuda et al. 2001), but the role of selfing vs. non-selfing systems, such as the mixed cleistogamous/chasmogamous mating system of *M. vimineum*, as a determinant of allelic frequency change and fixation (e.g., Allard and Workman 1963), should play a primary role in demographic determination for this species.

Patterns of genetic variation within and among invaded locations may offer clues about the relative importance of outcrossed vs. selfed seeds serving as founding propagules for new locations during range expansion. For example, if

there were some competitive advantage for highly homozygous propagules, established from cleistogamous seeds, then we might expect to observe lower genetic diversity in more recently colonized areas, relative to older populations. On the other hand, if highly heterozygous propagules, established from chasmogamous seeds, provide an advantage to founding propagules, then we might expect to observe higher genetic diversity in more recently colonized areas. As an empiric observation, it does not seem that either seed form is generically better or worse for *M. vimineum*. If it were, we should expect to see an evolutionary shift favoring whichever mating system provides that consistent advantage. There appears to be no evidence for this over the course of *M. vimineum*'s expansion in North America (Author's observations). The continued persistence of this mixed mating system implies tradeoffs having adaptive value for the species. Some advantage of each system to the plant's fitness (though the respective benefits for each mating system may be realized at distinct stages in the plant's life history) and may even be a major factor in its invasion success.

Several species exhibiting a mixed cleistogamous/chasmogamous syndrome exhibit patterns of genetic variation within populations that are typical of inbreeding species, but inter-population divergence (population structure) of these species can be more similar to that of out-crossing species. For example, in *Impatiens capensis* (Jewelweed), also an annual with mixed cleistogamous/ chasmogamous reproduction, mean within-population heterozygosity per individual was found to be low. Gene flow measures were low and genetic distances did not seem related to geographic distances, suggesting that

population structure was not continuous, but instead consisted of discrete demes exhibiting significant differentiation (Knight and Waller 1987).

If the mixed cleistogamous/chasmogamous syndrome is neither beneficial nor maladaptive enough to drive the mating system towards either cleistogamy or chasmogamy, the population structure may be dominated by random divergence, as a consequence of unpredictable founder effects. In this case, lack of global genetic diversity, or high global variation with no ecologically relevant pattern, would indicate that the mixed mating system may not be important in conferring invasive success. In addition to determining the importance of the cleistogamous/chasmogamous syndrome, population genetic study of this species will allow us to track future colonizers to their source localities and/or to adaptive habitat types.

I anticipate that the mixed cleistogamous/chasmogamous flowering syndrome is a major determinant of population genetic structure for *M. vimineum*. Initial establishment depends on seed transportation and subsequent colonization. Subsequent gene flow may also be accomplished by pollen flow. Based on what is currently known about the mixed mating system of *M. vimineum*, the plant generally invests more biomass in cleistogamous than in chasmogamous seed production (Gibson et al. 2002), though the allocation is mediated by both plastic and micro-evolutionary responses to light and other resource availability (Cheplick 2005, 2007). Since it is hard to imagine that the plant would be successful, especially as an annual, without some preservation (or even generation of) genetic variability to cope with changing and diverse

environments, it seems likely that occasional outcrossing due to chasmogamy may be useful to preserve some allelic variation, and even generate new allelic combinations, to counteract homogenization of populations resulting from inbreeding due to the plants' dominant cleistogamy.

I further expect that colonization of new habitats is most likely to result when the occasional seed with a novel genotype generated by chasmogamy is transported to a new locale, assuming those novel genotypes are either more fit in the new locale or particularly adept at dispersal. I therefore hypothesize that the species will exhibit low population level genetic diversity but relatively strong differentiation among populations (even in relatively close proximity), as preferential colonization of new habitats by chasmogamous seeds ought to favor population differentiation. Even 'neutral' marker loci such as microsatellites should reveal a signal of this process if they are linked to adaptively significant traits. This linkage seems likely for *M. vimineum* (given its high rates of inbreeding resulting from dominant cleistogamy), and has been noticed for other highly inbreeded grasses including *Bromus tectorum* (Ramakrishnan et al. 2004).

Objectives

I measured the genetic population structure and variability in both the native and invasive ranges of *M. vimineum,* using a newly developed battery of microsatellite (SSR) markers. I used the resulting patterns of genetic variability and structure to evaluate the mixed cleistogamous/ chasmogamous mating

system as a determinant of genetic structure for this species. I also attempted to discern the origin, within Asia, of *M. vimineum* propagules in the U.S., specifically by looking for Asian populations that are particularly closely related to U.S. populations. Finally, I attempted to determine the original location of *M. vimineum* introduction into the U.S. by hypothesizing that, similarly to crop plants, the center of diversity of a plant species should also be its center of origin and, possibly, the location of longest residence.

Materials and Methods

Populations Sampled

I collected *M. vimineum* samples from throughout its invasive range. I also obtained samples from China and Japan, courtesy of collaborators willing to collect the specimens, dry them, and send them to me in New Jersey. Sampling locations are depicted in Figs. 3.1 and 3.2 and listed in Table 3.1. In all, I collected 570 individuals from 34 populations. In the invasive range, I sampled only live plant materials from naturally occurring populations. I purposefully oversampled in and around Knoxville TN, so that I could evaluate whether that area, where *M. vimineum* was first recorded in the U.S., has more genetic diversity than other parts of the invasive range. Upon location of a population, I attempted to sample at least 20 individuals from along the longest transect through the population that I could access. I attempted to collect plants so that they were separated by at least one meter distance, in order to maximize the

genetic diversity sampled and to minimize the probability of collecting siblings. These same instructions were given to collaborators who collected in China. I was able to obtain samples from several populations in Yunnan Province, China, and from the area surrounding Shanghai from these collaborators. I was also able to obtain seeds collected from a population near Kyoto, Japan. These seeds were collected randomly from many individuals within the population to maximize genetic diversity and to minimize the fraction of siblings. I randomly germinated 25 of the Japanese seeds at Rutgers University (New Brunswick, NJ) and collected their leaves upon growing to sufficient biomass. All samples were dried in silica gel for transport and/or storage. Dried samples were pulverized with a mortar and pestle under liquid nitrogen, in preparation for DNA extraction, and then stored at -80°C at Rutgers University.

Molecular Assay for Genetic Structure Analysis

I extracted DNA from all samples with the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO), according to the manufacturer's specifications. I then amplified template DNA by PCR, using the protocol of Schuelke (2000), using microsatellite loci characterized for *M. vimineum* in Chapter 2 and later published (see also Novy et al. 2012, in press). Conditions of the PCR amplification were an initial heating of 94°C (5 min), followed by 30 cycles of 94°C (30 s)/56°C (45 s)/72°C (45 s), then 8 cycles of 94°C (30 s)/53°C (45 s)/72°C (45 s), and a final extension at 72°C for 10 min.

Each PCR reaction included the attachment of a fluorescent label (FAM, NED, PET, or VIC). I genotyped PCR products on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA), using a LIZ 500 size standard. I identified and binned alleles using GeneMapper 3.7 software (Applied Biosystems, Foster City, CA).

Genetic Structure of Populations

I scored the following microsatellite loci for 570 sampled individuals from 24 populations in the U.S. and 10 populations in Asia: MV01, MV02, MV03, MV05, MV06, MV07, MV08, MV09, MV10 (Chapter 2; Novy et al. 2012, in press). MV05 was clearly capturing two separate, and independent, loci which I scored separately and named MV05A and MV05B. I first analyzed the resulting allelic data in GenAlEx ver. 6.0 (Peakall and Smouse 2006) to generate summary data of allelic patterns, including heterozygosity and allelic distribution within and among populations. Heterozygosity is a widespread and biologically useful measure of genetic diversity in diploid species, since each individual is either homozygous or heterozygous at a given locus. However, to correct for variable sample sizes and provide an alternative estimate for genetic diversity, I calculated a bias-corrected effective number of alleles (Nielsen et al. 2003) in each population for comparative purposes. While heterozygosity is a traditional measure of genetic diversity in population genetics, Jost (2008) has shown that the effective number of alleles (here A_e^*) has standard numeric behavior and is a

more useful diversity measure. I conducted Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) to quantify population structure, using 999 permutations of the dataset to test for significance. I also conducted Principal Coordinates Analyses (PCO) in GenAlEx, which used a genetic distance matrix generated from the genotypic data, to determine whether observed patterns in the molecular data support the partitioning of the samples into specific groupings.

Based on the initial results of the PCOs and global AMOVA, with populations defined as sampling locations and regions defined as native or invasive, I conducted two additional AMOVAs of the native and invasive ranges separately, where I defined three regions within both Asia and North America. I also generated a range of *F*-statistics via AMOVA (F_{IS}, F_{SR}, F_{RT} and F_{IT}) to evaluate the relative importance of genetic variation at the individual, population and regional levels and a population by population matrix of pairwise F_{ST} values to test for population divergence. Jost (2008) has noted that G_{ST} (and by extension its analogue used here, F_{ST}), while a standard metric in population genetics, leaves much to be desired as measures of divergence. Therefore, I also calculated Jost's D (D_{est}) in the program SMOGD (Crawford 2010) to confirm the population divergences that I measured via AMOVA and F_{ST}. Finally, I used Bayesian clustering to attempt to determine the number of distinct population clusters (and the relationships between geographically determined populations) using the program STRUCTURE 2.3.3 (Pritchard, Stephens and Donnelly 2000, Falush, Stephens and Pritchard 2003, 2007, Hubisz et al. 2009). Estimates for *K* (the number of distinct population clusters), which are prior variables for the

program STRUCTURE, were generated by the method described by Evanno et al. (2005). Visualizations of STRUCTURE plots were generated in the program DISTRUCT 1.1 (Rosenberg 2004).

Results

Basic Population Genetic and Diversity Metrics

The nine microsatellite primer pairs yielded a total of 10 loci with 86 alleles, and amplified between 4 and 15 alleles per locus, with an average of 8.6 alleles per locus. Forty-nine alleles were found in North American populations and 71 alleles were found in Asian populations. Thirty-four alleles were shared among both Asia and North America. Observed heterozygosity (H_o) of sampled populations ranged from 0.00 to 0.16, expected heterozygosity (H_e) from 0.00 to 0.53, total number of alleles (N_a) over all loci for each population ranged from 10 to 26, and the effective number of alleles per locus (A_e^*) ranged from 1.00 to 2.55 (Table 3.2). I also generated a list of private alleles (i.e., alleles which appear in only one defined deme) for each locus assayed. In this case, I defined the demes as the invasive and native regions. The native region contained 37 private alleles, more than twice the number of private alleles found in the invasive region (15; Table 3.3).

In Asia, H_o was highest (0.16) at Shanghai Zoo, Shanghai, China. Half of the Asian populations sampled had an H_o of 0.00, including two locations in Zhe

Jiang Province, China; three in Shanghai Province, China; and Akabane, Japan. Three of ten (30%) populations had an A_e^* of 1.00 (its lowest possible value) in the native range.

In North America, H_o was 0.00 within all sampling locations except for Bloomington IN, Thurmont MD, Rockingham VA, and Morgantown WV. Eleven of 24 (46%) sampled populations had an A_e^* of 1.00 in the invasive range. Among those populations with $A_e^*>1$, Morgantown WV had the highest value (2.55). All calculated diversity metrics are presented by population sampled, as well as by regional and global average, in Table 3.2.

Globally, 25 out of 34 populations were genetically monomorphic and homozygous for all 10 loci. In order to more completely explore the nature of these highly homogenous and low diversity populations, I broke all homozygous and single locus heterozygotes into their respective haplotypes (Appendix Table A.1). This resulted in 108 separate haplotypes, 24 of which came from 12 single locus heterozygote diploid individuals. There were an additional eight individuals that were multilocus heterozygotes (Appendix Table A.2), for a total of only 20 individuals out of 570 assayed that were heterozygous at one or more loci. The vast majority of haplotypes were found in only one population. No haplotypes were found in both Asia and North America. In all, there were only 11 haplotypes (of 108) found in multiple populations. Of these, seven haplotypes were found in two populations, three were found in three populations and one was found in 16 populations. This one haplotype, found in 16 populations, was present in 229 samples, all from the southern U.S. and Indiana (Appendix Table A.1). The eight

multilocus heterozygote individuals came from five populations. Two of those populations were in West Virginia and three were from the Shanghai Zoo (Appendix Table A.2).

AMOVAs

I conducted three distinct AMOVA analyses (Table 3.4). In the first AMOVA, I defined each sampling location as a population and defined the invasive (USA) and native (Asia) sampling locations as the two regions. With this input, the AMOVA indicated that 22% of the variance was found among regions, 52% among populations within regions, 25% among individuals within single populations, and 1% within individuals (representing the 20, out of 570, heterozygous individuals). The AMOVA analysis generated several *F*-statistics to relate the various variance measurements including F_{RT} (among region variance/total variance), F_{SR} (among population variance/total variance within continents), F_{IS} (among individual variance/sum of the variances within and among individuals), and F_{IT} (sum of the variances among populations, regions, and individuals/total variance). For the first AMOVA, F_{RT} was 0.22, F_{SR} was 0.67, F_{IS} was 0.95, and F_{IT} was 0.99. For all *F*-statistics p = 0.001 based on permutational testing (Table 3.4, Fig. 3.3).

In the second and third AMOVAs, I treated the native and invasive regions separately. All populations were defined the same as for the first AMOVA, but I also defined three sub-regions within each of the invasive and native ranges, based on initial results from the PCO and STRUCTURE analyses (see below). In

the native range, I defined the regions as Japan, Yunnan, and the Shanghai area. In the invasive range, I defined the regions as northeast, mid-Atlantic/northern Virginia, and west/south. The exact population assignments for these regions are given in the **Discussion** (genetic structure sections). The results for the within-continent AMOVAs are presented in Table 3.4 and Fig. 3.4. The among region variance was much higher in the invasive (63%) than the native (16%) range. Consequently, since among individual and within individual variances were similar in both areas, the among population, within-region variance was much higher in the native (55%) than the invasive (14%) range. In *F*-statistical terms, F_{RT} was 0.16 in the native region and 0.63 in the invasive regions. F_{SR} was 0.66 in the native region and 0.39 in the invasive region (Table 3.4) indicating that the relative importance of sub-regional structure vs. population structure is greater in the invasive region.

PCOs

I generated three PCOs, based on the molecular dataset, in order to visually represent population and regional structure. For all PCOs, the first two coordinate axes captured over 50% of the total variance (total sum of the eigen values; Table 3.5). For the first PCO, I plotted all 570 samples by the first two principle coordinate axes and colored samples from the invasive (USA) and native (Asia) ranges differently (Fig 3.5). This PCO did reveal clustering of the Asian samples, though the entire cluster overlapped with samples from the USA range, reflecting the 34 shared alleles between the invasive and native ranges.

I also generated a PCO plot of the 123 Asian samples, for the first two principle coordinate axes, colored by population sampled (Fig. 3.6). In keeping with all of the results above, the three regions represented separate clusters. Populations from southwest China (the two populations from Yunnan Province), east China (the three populations from Zhe Jiang Province and four populations from Shanghai Province), and Japan (Nara Prefecture) were almost fully distinct, although there was slight overlap between some samples from Japan and one of the Shanghai Province populations. Furthermore, the southwestern China and eastern China clusters also exhibited separation (close clusters, but with minimal overlap) among the constituent populations.

I generated the third PCO using data from the 447 U.S. samples, and generated a plot of the first two principle coordinate axes, colored by population sampled (Fig. 3.7). There was minimal clustering of distinct populations, especially across regions. Instead, the dataset showed three 'spokes' of population clusters emerging from a diffuse central amalgam of samples from several populations. Broadly, the three 'spokes' could be classified as containing the northeast populations (New York, Connecticut and New Jersey), the mid-Atlantic/northern Virginia populations (Maryland, Pennsylvania and Rockingham VA), and the southern/western populations (both South Carolina populations, North Carolina, all Tennessee populations, Mecklenburg VA, Georgia, both Mississippi populations, Indiana, Alabama and Arkansas). All of the Ohio samples were located toward the center, near the intersection of the southern/western and northeast clusters. West Virginia samples did not cluster

with any one group and had samples toward the end of all three ‘spokes’. Populations from Rockingham VA, Mecklenburg VA, Georgia, Alabama and Arkansas, Holly Springs MS, both populations from South Carolina, all populations from Tennessee, New York and Connecticut were wholly contained within their respective ‘spokes’. Samples from the remaining populations were mostly contained within their respective ‘spokes’ but contained at least one sample located in another ‘spoke’ or in the diffuse center.

Pairwise Population Divergence

In order to quantify divergence between populations, I generated two types of population x population pairwise matrices. Table 3.6 shows a matrix of pairwise population F_{ST} values calculated from AMOVA. Numbers below the diagonal are the F_{ST} values and numbers above the diagonal are P-values in support of the corresponding F_{ST} value, based on permutation testing. Insignificant values have been colored yellow. All Asian populations were significantly differentiated. Interestingly, two of the populations from Shanghai Province, China were not significantly differentiated from populations from Alabama, two of the Tennessee populations, and one of the South Carolina populations. None of the Tennessee populations were significantly differentiated from each other. Alabama was not significantly differentiated from Arkansas, Indiana, one of the Mississippi populations, and all but one of the Tennessee populations. Arkansas was not significantly differentiated from most of the

southern/western populations seen in the PCO of U.S. samples. Georgia was not significantly differentiated from Indiana, most of the Tennessee populations and one of the Mississippi populations. Holly Springs MS was not significantly differentiated from Mayo SC and Mecklenburg VA. All other populations were significantly differentiated. Of the significant pairwise population relationships, F_{ST} values ranged from 0.00 to 0.968 indicating varying levels of divergence. In the vast majority of cases, pairwise F_{ST} values were less than 0.500 for samples from the same regions within North America, further lending support to the relationships visualized in the PCO.

Since Jost (2008) noted that G_{ST} (and by extension its analogue used here, F_{ST}) is a less than ideal measure of differentiation between demes, and one that can succumb to various estimation errors, I also generated a pairwise matrix of the harmonic mean of Jost's D_{est} (Table 3.7), which should be a superior measure of divergence between demes. This measure showed the same trends as did F_{ST}, but with the following exceptions. One Shanghai province did not show divergence from the Maryland populations. One Zhe Jiang Province population also did not show divergence from the Maryland population. All other Asian populations were divergent from each other although the two Yunnan and two of the Zhe Jiang Province populations showed very low levels of divergence (<0.100). In the invasive range, populations from Alabama, Arkansas, Georgia, Indiana, all Tennessee populations, both Mississippi populations, Mayo SC, and Mecklenburg VA showed no divergence from each other. In addition, the North Carolina population showed no divergence from the Mayo SC, and Mecklenburg

VA populations. All other U.S. populations were differentiated but similarly to the F_{ST} results were consistently less differentiated among the regions indicated in the PCO. Though the only Asian populations which showed no divergence from some North American populations were from eastern China, Japanese populations were, on average, less divergent from the North American populations than other Asian populations. For these populations D_{est} ranged from 0.234-0.416. There were also several populations from eastern China which had lower differentiation from several North American populations. For these populations D_{est} ranged from 0.345-0.550. Populations from different sub-regions within each continent generally had a D_{est} greater than 0.600. Interestingly, D_{est} values generally showed less divergence of the southern/western North American populations with the Japanese population while the remainder of North American populations showed less divergence with eastern Chinese populations.

Bayesian Clustering via STRUCTURE

I also attempted to resolve genetic structure using the Bayesian clustering program STRUCTURE 2.3.3, although I should note that the STRUCTURE analysis results should be interpreted carefully since the STRUCTURE algorithms assume Hardy-Weinberg equilibriums within populations, which is not a valid assumption for *M. vimineum*, due to high rates of inbreeding. Nonetheless, I conducted the STRUCTURE analysis as an exploratory exercise to determine what kind of sub-regional genetic structure may be revealed by a Bayesian approach and to generate a non-numerical, visual representation of the

data. One of the critical decisions in setting the STRUCTURE input is deciding a reasonable value for K, the expected number of clusters. Using the Evanno et al. (2005) method, I compared K vs. DeltaK values for 20 runs of the dataset for various K values. DeltaK is an *ad hoc* statistic that quantifies the rate of change in the log probability of data between successive K values. I initially ran the analysis for K = 2 to K = 26, based on STRUCTURE runs set for 10,000 burnin reps and 10,000 MCMC (Marcov Chain Monte Carlo) replications. Based on the Evanno et al. (2005) method, the optimal K value should be indicated by plotting K vs. DeltaK, looking for a high peak of DeltaK and then adopting the corresponding K value. This would clearly be K = 6 based on the graph presented in Appendix Figure A.1.A. I also plotted K vs. DeltaK for K = 2 to K =19, based on STRUCTURE runs set for 50,000 burnins and 200,000 MCMC reps, which is well within the range of STRUCTURE simulations run parameters used for publication quality results. Strangely, the K vs. DeltaK results were much more ambiguous with the additional burnins and MCMC reps (Appendix Figure A.1.B). The peak DeltaK appeared to be at either K = 4 or K = 9. Because of these ambiguities, and because choosing a K value is considered as much an art as a science (STRUCTURE 2.3.3 support manual), I generated a range of STRUCTURE graphs, visualized using DISTRUCT 1.1 to explore the simulations. I present the results from three STRUCTURE simulations of K = 6 and K = 7, generated using 10,000 burnins and 10,000 MCMC reps in Appendix Figures. A.2 and A.3. I also present the results from three simulations of K = 5, K = 9 and K = 11 of 50,000 burnins and 200,000 MCMC reps in Appendix Figures

A.4-A.6. However, since *M. vimineum*'s reproductive biology is not fully compatible with STRUCTURE analysis, I do not go into their results nor interpret their implications here.

Discussion

Genetic Diversity and Inbreeding

I initially hypothesized that *M. vimineum* would exhibit low within population genetic diversity based on its mating system. This certainly appears to be the case. The majority of populations exhibited $H_0 = 0.00$, indicating fixation of alleles (or maintenance of additional alleles at lower frequency than were detectable by my sample sizes) for all of the microsatellite loci assayed here in the majority of populations. This pattern was evident in both native and introduced populations of the species, suggesting that the pattern is not solely due to bottlenecks associated with invasion, but rather a general property of the species. This pattern has almost certainly emerged as a result of the high selfing rate inherent in the mixed cleistogamous/chasmogamous breeding system. As a species which is wind pollinated, has no known self-incompatibility, and seems to have ample opportunity for seed dispersal, inbreeding due to cleistogamy appears to be the most plausible explanation of the extremely low levels of within population diversity observed here. The fact that so many of the populations exhibited allelic fixation, or low levels of genetic diversity, despite tremendous abundance and sustained invasion success, suggests that the reduced genetic

diversity within populations accompanying high selfing rates is not detrimental to the species' survival.

While inbreeding is undoubtedly an important determinant of population structure in this species, the lack of internal variation may also reflect my choice of marker system. This is best indicated by comparing the results from this study with the genetic analysis performed of invasive *M. vimineum* in a single watershed in Virginia, using AFLP markers. In that study, Baker and Dyer (2011) did not find complete fixation within populations, though STRUCTURE analysis did indicate that all but two of their 23 populations showed little indication of admixture, which could mean that AFLP locus diversity has been generated *de novo* in each population, as opposed to resulting from gene flow. They measured mean diversity (Shannon's information) at 0.264, ranging from 0.148 to 0.380 among populations, although they were unable to measure heterozygosity, since AFLP is a dominant marker system. They also measured percent polymorphic loci, which ranged from 19.44% to 77.78% with a mean of 47.94%. The Baker and Dyer (2011) results, indicate that there is more genetic variation within populations, at least when assayed via AFLP, than is revealed by the microsatellite marker system used here.

Notwithstanding this dearth of within-population variation, the microsatellite marker system did uncover important genetic structure at both the regional and continental scales. Therefore, future studies of *M. vimineum* at larger landscape scales would benefit from this microsatellite marker system, while studies at a more local scales (within watersheds or individual populations)

will require genetic markers capable of discerning greater variability (e.g., AFLPs, SNPs or ISSRs). Since accurate measurement of heterozygosity is likely to be of high value for this species, SNPs (a co-dominant marker system capable of detecting heterozygosity directly) might be the better choice.

Genetic Structure in the Native Range

There were several clear indications of spatially determined genetic structure in the native range. Pairwise F_{ST} values were significant and high between all populations in the native range. Similarly, pairwise values of Jost's D_{est} were also high between most populations in the native range. Both the AMOVA and PCO analyses clearly revealed clustering of populations based on large-scale geography. Though this study includes populations from only a small portion of the species' native range, which extends westward to Iran, northward to Russia and southward to Myanmar, it is evident that the species exhibits genetic subdivision on a transcontinental scale, within its native range. Additional sampling would allow for a more complete biogeographic analysis of the species within its native range and help identify geographic barriers to gene flow which may be important determinants of finer scale genetic structure in the native range. Such sampling may also provide more genotypes which could be used as comparators to help identify the most likely source(s) of propagules giving rise to invasive *M. vimineum* populations.

Genetic Structure in the Invasive Range

Microstegium vimineum showed clear indications of genetic structure in its invasive range, but the patterns of genetic structure were clearly different from those found in Asia. While many pairs of populations were significantly different, based on pairwise F_{ST} (or D_{est}) values, others were not. In broad terms, all analyses showed that populations within the invasive range can be broken up into three sections: the northeast (New York, New Jersey and Connecticut), the south/west (Alabama, Arkansas, Georgia, Indiana, Tennessee, Mississippi, South Carolina, and southern Virginia), and the mid-Atlantic (Maryland, Pennsylvania and northern Virginia). Beyond broad scale partitioning, it was clear that the populations within these sub-regions were not as well differentiated based on geography as their counterparts in Asia. In other words, populations within these sub-regions were less differentiated *inter se*. This is also evidenced by the lower F_{SR} value in the invasive range than in the native range (Table 3.4).

I deliberately oversampled from populations in and around Knoxville, Tennessee, in order to examine the genetic diversity at the first recorded sampling location for the species in its invasive range. Often, species show the highest genetic diversity where they have been present longest, as with domesticated crop species (Vavilov 1951), though I must admit the caveat that herbarium records are not always a reliable proxy for relative dates of first presence. For *M. vimineum* there was no increase in genetic diversity in the Knoxville region and, in fact, all Tennessee populations were virtually indistinguishable. This may indicate that genetic diversity is quickly purged within

M. vimineum populations, as is usual from repeated generations of high selfing rates. Of course, it could also represent the consequences of a highly restricted, genetically depauperate source of colonizing propagule(s) from Asia. In either case, it may not be possible to determine the oldest, or original, invasion locale(s) by seeking out areas with increased genetic diversity in the invasive range.

Based on anecdotal accounts, the prevailing notion is that *M. vimineum* was first introduced to the southeastern U.S. around 1900. The fact that this study revealed three distinct, divergent groupings within U.S. populations could be interpreted as suggesting three introductions of distinct genetic material from Asia, giving rise to the three different geographic groupings discovered here. The herbarium records for the species do not seem to corroborate that possibility. Instead, the herbarium records suggest that the species was probably introduced in the southeastern U.S., with potential secondary introduction location(s) in the mid-Atlantic, based on the early appearance of specimens around Philadelphia (Fairbrothers and Gray 1972). Considering that the species was reported to be introduced via packing material from Chinese ceramics, it is mostly likely that the species would have been introduced multiple times wherever these ceramics packages were opened and the packing materials discharged. However, it is possible that the introduced material would have been genetically similar, since most Chinese ceramics imports originated from the Janxi Region in central China.

Most likely, the founding invasive propagules would have been cleistogamous (and thus homozygous) since the terminal, chasmogamous seeds shatter under field conditions and would probably have fallen off the plant before harvesting. However, genetic diversity could still have been introduced into the invasive range via multiple introductions of differing homozygous material. Both the available 'lore' and the pattern of genetic structure in the invasive range suggest multiple introductions (from central China) to the southeastern (and possibly mid-Atlantic) United States. Under such a scenario, range expansion southwards, northwards and westwards would have resulted in genetic radiation, giving rise to the three genetic sections observed in this study. It is interesting to note that populations from Ohio and West Virginia defied classification, relative to the three observed sub-regions. West Virginia also had the highest genetic diversity of any population measured by effective number of alleles. It may be that the northeastern mid-west represents a secondary contact zone where expansion of the northeast sub-region westward is converging with expansion of the southern/western sub-region northward. This interpretation is further supported by the observation that these populations are some of the most recently established. As I initially anticipated, higher diversity in West Virginia and Indiana could also indicate an advantage of increased chasmogamy in more recently established populations, though comparatively higher levels of diversity were not noticed for other young populations (e.g., New York and Connecticut). Alternatively, these recent arrivals into the heart of the continent may represent novel genotypes introduced anew from the native range via international shipping

up the Mississippi and then branching off to the Ohio. Introduction trade routes branching off the St. Lawrence or even up the Susquehanna (via the Chesapeake Bay) are also possibilities. Whether, and how, *M. vimineum* propagules are still being transported to the U.S. from Asia is likely the key determinant for which scenario is most probable.

Relationship of the Invasive Range to the Native Range

Invasion biologists are often curious as to the origin and genetic diversity of colonizing propagules. Large scale population genetic analyses which include native and invasive populations may sometimes reveal the origin(s) of colonization, and later invasion. Looking through the data, there are a few lines of evidence that the populations from eastern China may be slightly more similar to some of the U.S. populations than the other Asian locales sampled, though the evidence is mixed. First and foremost, there is substantial allelic overlap between the two continents (34 of 86 total alleles) indicating that genetically, the two continents have quite a bit in common. Additional evidence stems from the pairwise measures of differentiation. The F_{ST} analysis revealed that there were four populations from the southern U.S. which were not differentiated from Asian populations (F_{ST} = 0.00). In all of these cases, these relationships were with populations from eastern China. The D_{est} analysis also revealed similar relationships although additionally revealed a signature that some North American populations appeared less divergent from the Japanese population

than from eastern Chinese populations. However I do not find it credible that U.S. populations are more closely related to Japanese populations than Chinese populations. I have grown Japanese and Chinese *M. vimineum* plants in the greenhouse. Though I had access to limited sampling locations, all Japanese plants exhibited yellow anthers. All North American plants exhibited reddish brown anthers. Most Chinese populations contained both yellow and reddish brown anthers. This visual marker data (presumably under genetic control) would seem to indicate that North American *M. vimineum* originates from somewhere in the native range with the presence of reddish brown anthers, which at least according to my limited sampling does not include Japan.

Collectively, the information can be interpreted in three ways. First, it could mean that overall, the eastern Chinese samples are slightly more similar to the U.S. samples than the other Asian populations. This interpretation does compliment the anecdotal account that *M. vimineum* was introduced via packing material used for shipping of Chinese porcelain, which was primarily imported from Janxi province around the turn of the century. I was unable to obtain samples from Jianxi province for this study, but since Janxi is located between Shanghai and Yunnan, but closer to Shanghai, it does make sense that the dataset would reveal a weak, yet somewhat ambiguous, signal of similarity between American and eastern Chinese samples. Alternatively, the evidence could indicate that I have not sampled the source of *M. vimineum* in the Asian range at all. The species is present in areas quite geographically distinct from my current Asian sampling locations, including the Philippines, Myanmar, India and

Russia. Any of these locations could contain vastly different genetic versions of *M. vimineum* which may have found their way to North America, though the substantial allelic overlap between the two regions sampled here does suggest that I may have sampled in (or near) the source of invasive propagules. Third, the lack of haplotypic similarity between continents could be the result of divergence over the species' 100 or more year history in North America. We have no strong indication whether the species was introduced to a single location, or has been continuously reintroduced from one or several Asian sources. Any of these scenarios could explain the intercontinental divergence seen here between Asia and North America. Furthermore, since these are nuclear markers which can assort independently, and certainly would over 100 generations, there could have been a large amount of genetic reshuffling occurring in the invasive range, which could differentiate those populations from any Asian source populations. Although repeated selfing due to cleistogamy would link the markers, resulting in non-independent assortment, the clear presence of occasional chasmogamy (eight multilocus heterozygotes in 570 samples) could still provide fodder for independent assortment.

Even though I cannot make any definite statements about invasive propagule origin based on this dataset, there are some important general differences in overall genetic diversity between the native and invasive ranges that are evident. First, the overall genetic structure of populations is partitioned on different spatial scales. In Asia, there is clear differentiation between the sub-regions sampled. These sub-regions appear almost completely distinct, based on

the PCO analysis. Furthermore, all populations, even those collected in relatively close proximity (e.g., in Yunnan and the Shanghai sub-regions), are significantly and robustly differentiated. In contrast, the North American samples show a more subtle sub-regional structure, but are not well differentiated within sub-regions. Furthermore, those sub-regions are not as clearly discrete, as there are populations (e.g., West Virginia) that clustered with multiple sub-regions in the PCO. This suggest that in the invasive range, the migration rate of microsatellite loci (*m*; within a region) is greater than the mutation rate (μ), whereas in Asia, *m* (within a region) < μ. The fact that there is one haplotype shared by 16 populations, and 229 individuals, in North America is certainly strong evidence that that particular haplotype is dispersing around the invasive range much more quickly than it is evolving.

The natural interpretation is that the genetic structure in the invasive range continues to 'sort itself out' as the species continues to colonize its new range. Given enough time, I would expect that the invasive range populations would differentiate as fully as those in the native range, once range expansion into suitable niches is complete and the new populations suffer the expected 'meltdown' of their starting genetic variation due to the inbreeding that accompanies repeated selfing, coupled with the eventual generation of new microsatellite alleles mutationally in each population.

Though I am not sure that the Asian populations sampled are representative of potential source populations for the invasive range, it is very evident that the North American samples contain less genetic diversity than do

the Asian samples, probably as a result of the bottleneck often associated with invasion. There are two lines of evidence to suggest this bottleneck. First, measured averages of heterozygosity and effective numbers of alleles were higher in the native range. This is especially noteworthy, since I sampled fewer populations (10) in the native range than in the invasive range (24), and the average sample size for Asian populations ($\tilde{n}_A$ = 12.3) was lower than in the U.S. ($\tilde{n}_U$ = 18.6). Everything else being equal, we would have expected the reverse results, so the fact that $A_{e(A)}^* > A_{e(U)}^*$ is striking. Second, there were more than twice the number of private alleles in the native range (Table 3.3), suggesting greater allelic diversity there.

Importance of the Mating System

This study serves as a strong reminder that the biology of the organism, especially the reproductive biology, is likely to be the most important feature in determining broad scale genetic structure. While I did find some interesting differences in both genetic diversity and population genetic structure between the native and introduced ranges, individual populations in the native and invasive ranges were remarkably similar. They exhibited the predicted patterns associated with an annual, wind pollinated grass with a mixed cleistogamous/ chasmogamous mating system. Diversity was low within all populations, and there was definite genetic structure on regional levels. Since *M. vimineum* is a successful invader, it is apparent that the mixed cleistogamous/chasmogamous

mating system is not hampering the species' ability to cope with its new environment. In fact, it is entirely possible that this strategy is beneficial to the organism. Though this kind of reproductive strategy would result in narrowing of genetic diversity in each breeding population, due to the increased rates of inbreeding, high rates of inbreeding would not necessarily lead to the negative fitness consequences usually associated with exposure of detrimental recessive alleles, because high rates of inbreeding would have already purged the species of such deleterious alleles.

I initially expected that I would be able to infer an adaptive advantage of the mixed cleistogamous/chasmogamous mating system based on patterns of genetic structure. I hypothesized that novel genotypes generated by chasmogamous outcrossing would lead to strong differentiation among populations (even in relatively close proximity) if novel genotypes are either more fit in new locales or particularly adept at dispersal. The observed sub-regional structure within the invasive range, and lack of population differentiations within sub-regions (e.g., the entire southern U.S. and especially the greater Knoxville area), despite clear differences in habitats within these regions, does not support this specific hypothesis based on the data patterns observed. However, the lack of population differentiation does not necessarily mean that the mixed cleistogamous/chasmogamous mating system is not generating novel colonizing propagules in this way. Instead, these microsatellite markers may be behaving more 'neutrally' than I originally anticipated. It appears that even a small amount of outcrossing (about 3.5% of individuals were heterozygous in this study) may

be sufficient to break marker linkage with potentially adaptive genes. Therefore, the genetic structure observed in this study is likely to be more related to overall demographic processes than to adaptive qualities.

Nevertheless, this study provides empirical evidence that the mixed cleistogamous/chasmogamous mating system allows for the near fixation of genotypes in a given habitat, while the chasmogamous terminal spike present on each plant, and the occasional outcrossing that it allows, leads to persistence of some genetic diversity at low frequency, including generation of novel allelic combinations (20 of 570 samples showing some sign of outcrossing), though continued inbreeding would slowly erode allelic diversity, absent other pressures or gene flow. These demographic qualities are compatible with adaptationally significant processes. For example, the maintenance of low frequency alleles would serve as a reservoir of genetic diversity that could quickly increase frequency under appropriate selection pressure. Therefore, *M. vimineum* may be leveraging the mixed cleistogamous/chasmogamous mating system to advantage, using the system to episodically create novel genotypes in a given environment, followed by fixation of the better adapted recombinants via inbreeding. Since Cheplick (2007) found that biomass allocation to chasmogamy increased relative to cleistogamy under only the most favorable growing conditions, there does appear to be some cost to increasing investment in chasmogamy for this species. This cost could reflect pressure on the species to reduce the possibility of deviation from ‘fixed’ genotypes, which have developed adaptationally at a given site, except when conditions are particularly favorable to

survival. Thus novel genotypes (which could include genotypes especially fit for colonization of new sites but would probably include some expensive (i.e., unfit) genotypes will be preferentially generated at times when reproductive allocation to unfit genotypes would present less of an investment risk to survival. Furthermore, the divergence between sub-regions which has developed post-colonization, may serve as an additional reservoir of genetic diversity that may provide capitalization opportunities for adaptive processes during range expansion. Since seeds are easily dispersed, new genetic diversity should be flowing between populations (though at rates low enough to allow inbreeding to 'fix' populations for genotypes as was observed in this study) and would be available should resource availability present the opportunity.

Conclusions

The mating system of *M. vimineum* is the most important determinant of the continental and regional level population structure observed in this study, though there were some differences evident in population structure between the invasive and native ranges. Specifically, the invasive range had lower genetic diversity, overall, probably due to founder effects. Also, in its invasive range, population and regional genetic differentiation appeared to be 'in process' of developing, due to the relative importance of migration to mutation in the invasive range as compared to the native range. Sub-regional structure among populations in the invasive range has been established and will probably move

towards the level of divergence that is evident in the native range. Divergence and differentiation in the invasive range are likely to continue as the species expands its invasive range, generates new diversity mutationally in the new range, and (possibly) via additional introduction of genetically distinct propagules from the native range. Continued population genetic studies of *M. vimineum*, especially those using co-dominant and highly polymorphic marker systems (e.g., SNPs), will likely elucidate the time scales under which the processes which determine genetic structure operate and provide more information about the exact locations in the native range which have served as sources for invasive propagules.

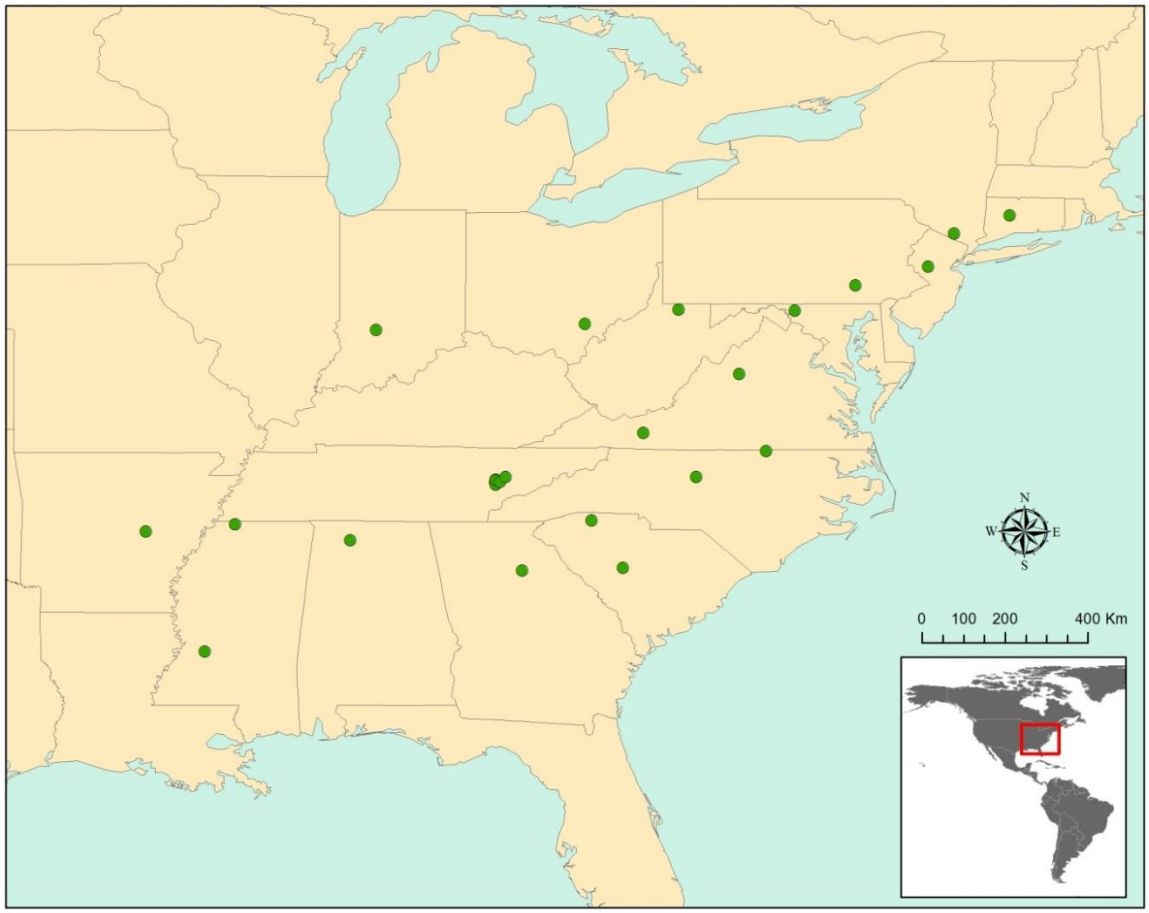


Figure 3.1. Population sampling locations from the United States used in population genetic analyses of *Microstegium vimineum*.

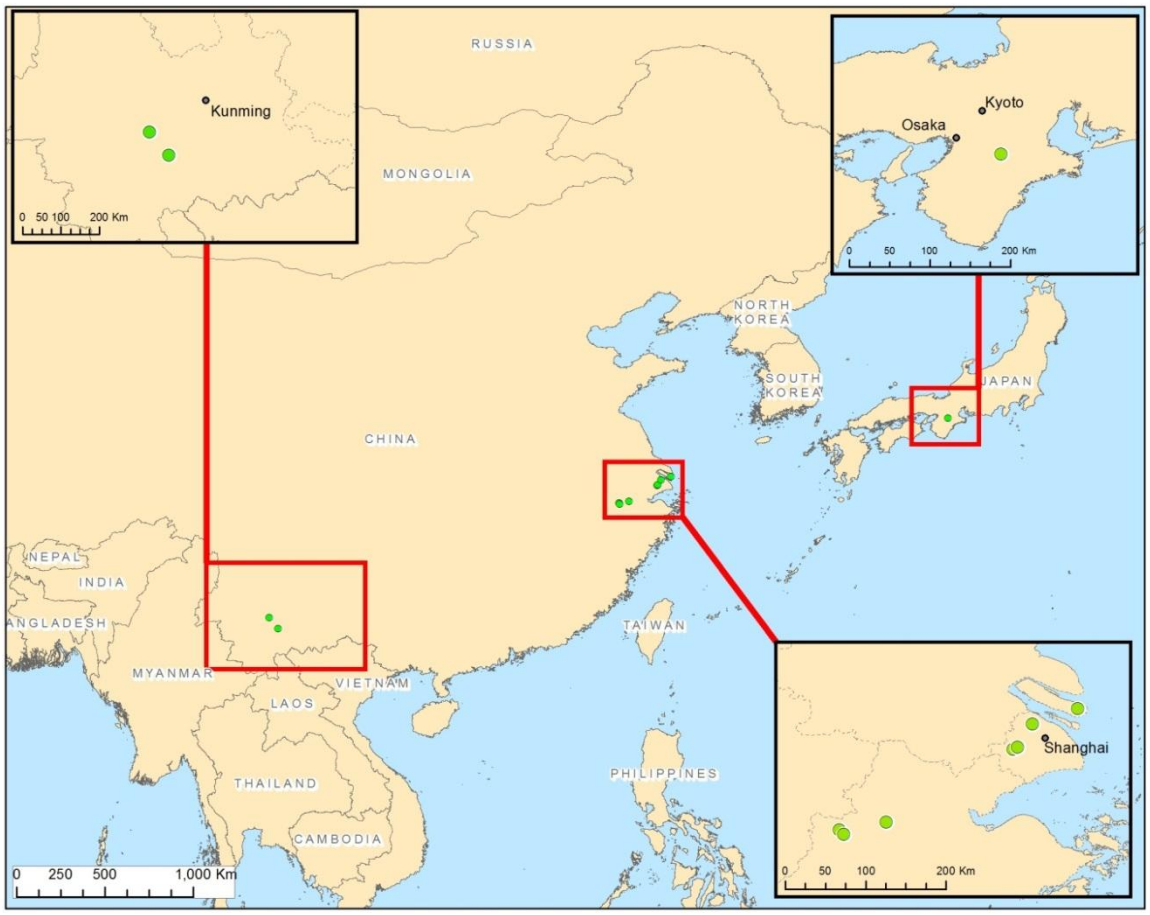


Figure 3.2. Population sampling locations from Asia used in population genetic analyses of *Microstegium vimineum*.

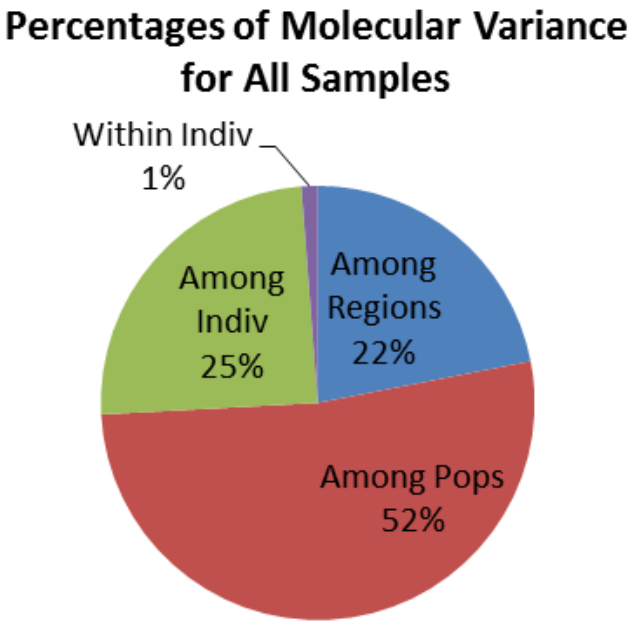


Figure 3.3. AMOVA chart and summary statistics for all *M. vimineum* samples with regions defined as Asia and the USA.

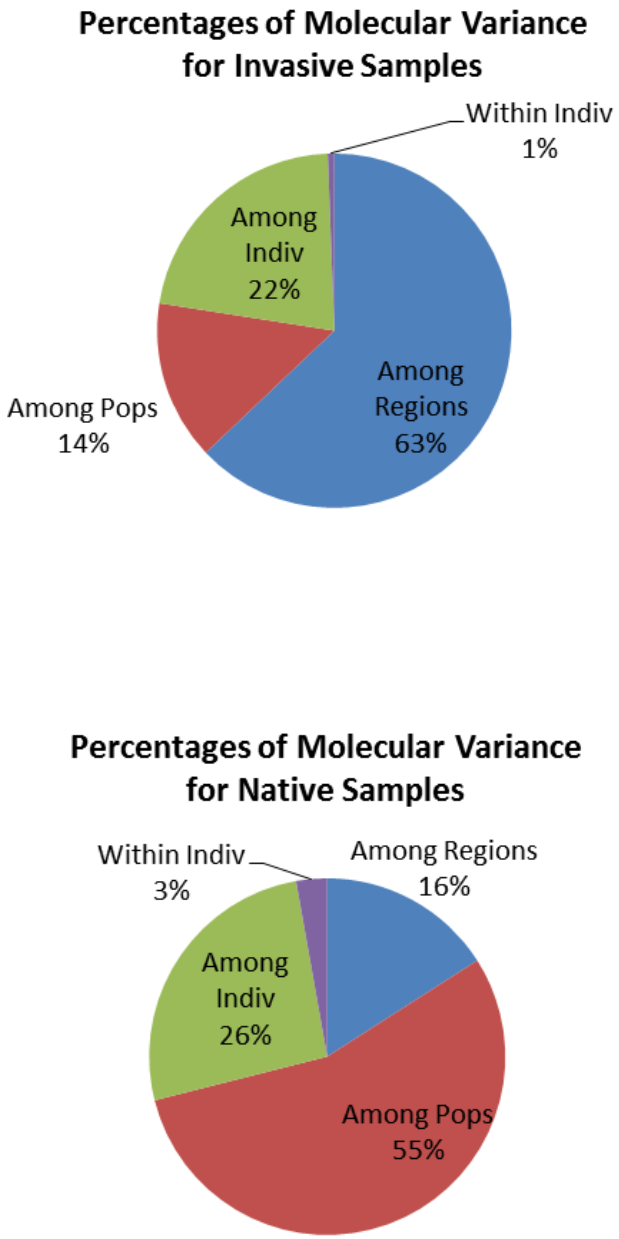


Figure 3.4. AMOVA chart for *M. vimineum* samples from the invasive (USA) and native (Asia) ranges separately. Ranges in the US are defined as north east, mid-Atlantic/north Virginia, and the west/south. Regions for Asia are Yunnan, the Shanghai region, and Japan.

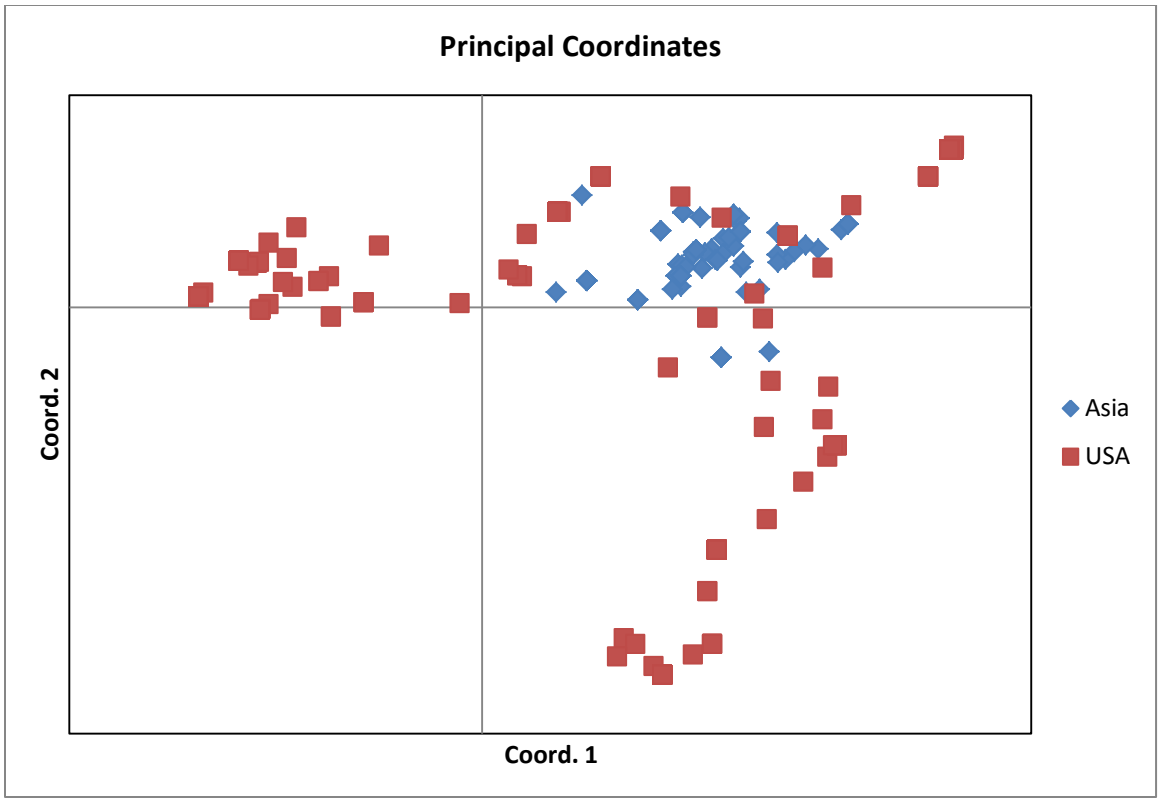


Figure 3.5. Principle Coordinate Analysis (PCO) of all *M. vimineum* samples colored by region (Asia and USA).

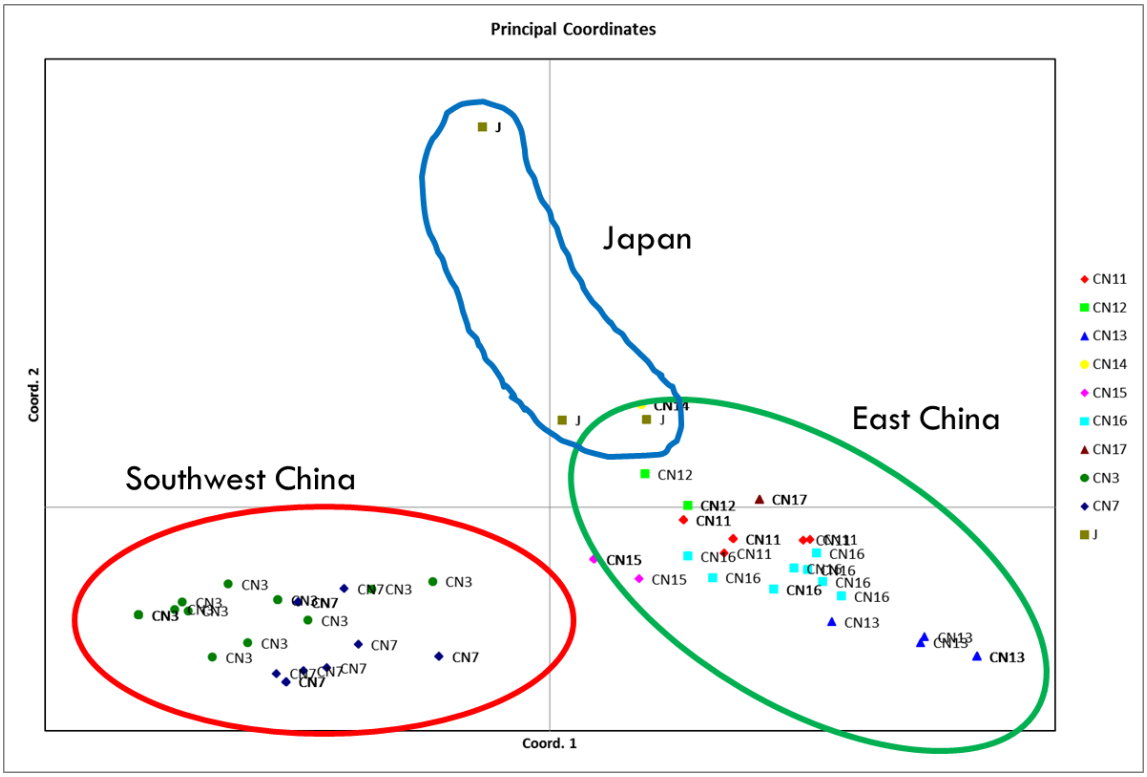


Figure 3.6. Principle Coordinate Analysis (PCO) of Native (Asian) samples colored and labeled by population. Colored groupings indicate geographic origins of each sample within Asia.

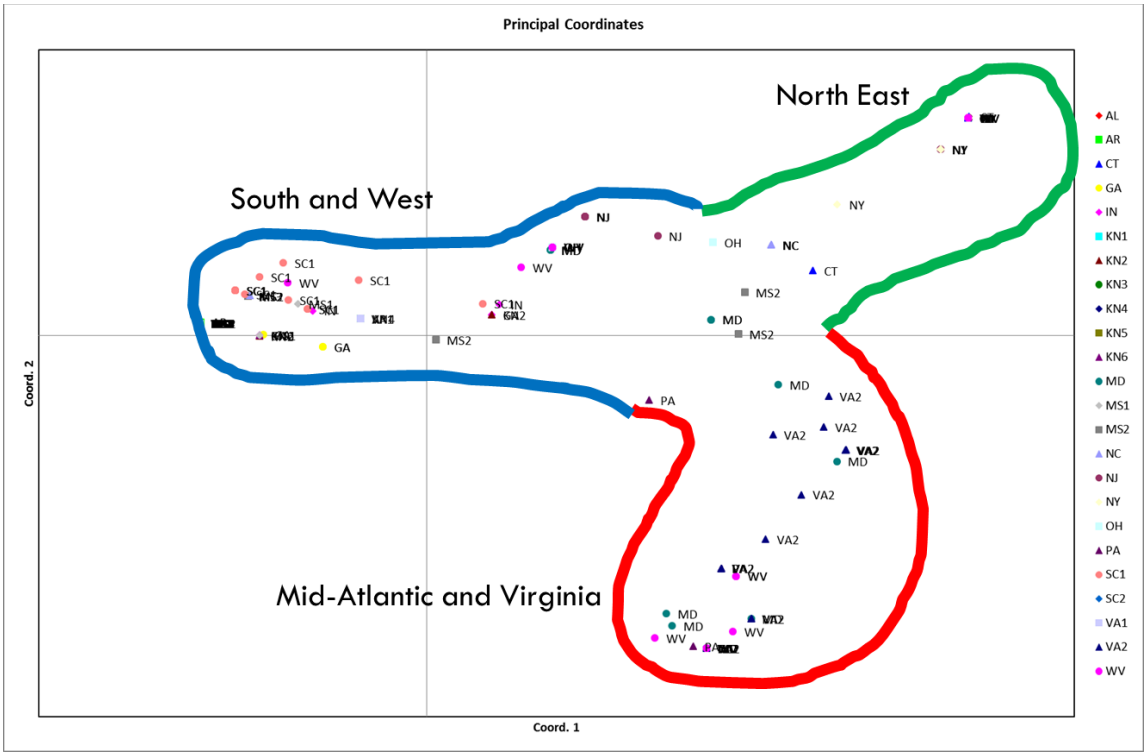


Figure 3.7. Principle Coordinate Analysis (PCO) of invasive (USA) samples colored and labeled by population. Colored groupings indicate geographic origins of each sample within the U.S.

Population Code	Country	State/Province	Nearest town/Landmark	Latitude	Longitude
CN11	China	Zhe Jiang	Qingliangfen Reserve	30° 10' 29" N	119° 11' 56" E
CN12	China	Zhe Jiang	Yunlang Village	30° 07' 17" N	119° 14' 37" E
CN13	China	Zhe Jiang	Zhe Jiang A&F University	30° 15' 24" N	119° 43' 22" E
CN14	China	Shanghai	Tianma Mountain	31° 04' 35" N	121° 08' 41" E
CN15	China	Shanghai	She Mountain	31° 05' 51" N	121° 11' 26" E
CN16	China	Shanghai	Shanghai Zoo	31° 21' 34" N	121° 21' 34" E
CN17	China	Shanghai	Chongming Island	31° 31' 52" N	121° 51' 52" E
CN3	China	Yunnan	Mosha	23° 45' 37" N	101° 48' 45" E
CN7	China	Yunnan	Zhelong	24° 18' 10" N	101° 21' 50" E
J	Japan	Nara Prefect	Akabane	34° 30' 51" N	136° 00' 38" E
AL1	USA	Alabama	Decatur	34° 38' 57" N	87° 06' 20" W
AR1	USA	Arkansas	Hazen	34° 50' 23" N	91° 33' 15" W
CT1	USA	Connecticut	Farmingham	41° 42' 22" N	72° 47' 34" W
GA1	USA	Georgia	Athens	33° 59' 22" N	83° 22' 46" W
IN	USA	Indiana	Bloomington	39° 13' 09" N	86° 32' 29" W
KN1	USA	Tennessee	Knoxville	35° 56' 54" N	83° 56' 21" W
KN2	USA	Tennessee	Knoxville	35° 57' 19" N	83° 56' 50" W
KN3	USA	Tennessee	Knoxville	35° 54' 14" N	83° 57' 41" W
KN4	USA	Tennessee	Maryville-Alcoa	35° 51' 12" N	83° 56' 47" W
KN5	USA	Tennessee	Knoxville	35° 54' 44" N	83° 51' 19" W
KN6	USA	Tennessee	Knoxville	36° 00' 55" N	83° 44' 23" W
MD1	USA	Maryland	Thurmont	39° 37' 46" N	77° 27' 32" W
MS1	USA	Mississippi	Holly Springs	34° 59' 14" N	89° 36' 33" W
MS2	USA	Mississippi	Jackson	32° 13' 29" N	90° 15' 58" W
NC1	USA	North Carolina	Rock Creek	36° 01' 22" N	79° 35' 24" W
NJ1	USA	New Jersey	Bridgewater	40° 35' 19" N	74° 33' 47" W
NY1	USA	New York	Bear Mt.	41° 18' 30" N	74° 00' 01" W
OH	USA	Ohio	Athens	39° 20' 30" N	82° 00' 47" W
PA1	USA	Pennsylvania	Ephrath	40° 10' 49" N	76° 08' 22" W
SC1	USA	South Carolina	Columbia	34° 02' 57" N	81° 10' 58" W
SC2	USA	South Carolina	Mayo	35° 04' 14" N	81° 52' 29" W
VA1	USA	Virginia	Mecklenburg	36° 34' 42" N	78° 04' 23" W
VA2	USA	Virginia	Rockingham	38° 15' 43" N	78° 39' 40" W
WV	USA	West Virginia	Morgantown	39° 39' 45" N	79° 59' 00" W

Table 3.1. Population codes and sampling locations of all *M. vimineum* samples used in the population genetic analysis.

Population Code	Country	*N*	H_o	H_e	N_a	A_e^*
CN11	China	11	0.07	0.35	25	1.65
CN12	China	7	0.00	0.02	11	1.04
CN13	China	13	0.01	0.01	11	1.01
CN14	China	8	0.00	0.00	10	1.00
CN15	China	7	0.00	0.00	10	1.00
CN16	China	10	0.16	0.32	22	1.67
CN17	China	5	0.00	0.00	10	1.00
CN3	China	20	0.01	0.16	16	1.28
CN7	China	20	0.01	0.29	21	1.67
J	Japan	22	0.00	0.19	21	1.28
AL1	USA	24	0.00	0.00	10	1.00
AR1	USA	20	0.00	0.00	10	1.00
CT1	USA	15	0.00	0.00	10	1.00
GA1	USA	16	0.00	0.05	12	1.08
IN	USA	22	0.01	0.00	11	1.01
KN1	USA	7	0.00	0.00	10	1.00
KN2	USA	10	0.00	0.02	11	1.02
KN3	USA	15	0.00	0.01	11	1.02
KN4	USA	19	0.00	0.00	10	1.00
KN5	USA	11	0.00	0.00	10	1.00
KN6	USA	5	0.00	0.00	10	1.00
MD1	USA	23	0.03	0.35	26	1.64
MS1	USA	20	0.00	0.00	10	1.00
MS2	USA	25	0.00	0.12	21	1.16
NC1	USA	25	0.00	0.36	23	1.67
NJ1	USA	18	0.00	0.40	18	1.86
NY1	USA	21	0.00	0.00	10	1.00
OH	USA	20	0.00	0.00	10	1.00
PA1	USA	23	0.00	0.19	14	1.38
SC1	USA	24	0.00	0.04	14	1.05
SC2	USA	18	0.00	0.00	10	1.00
VA1	USA	22	0.00	0.00	10	1.00
VA2	USA	24	0.01	0.34	22	1.94
WV	USA	20	0.03	0.53	28	2.55
Global Average		16.76	0.01	0.11	14.35	1.23
Asia Average		12.30	0.03	0.14	15.70	1.26
USA Average		18.63	0.00	0.10	13.79	1.22

Table 3.2. Genetic diversity metrics for *M. vimineum* populations sampled. *N* = number of samples, H_o = observed heterozygosity, H_e = expected heterozygosity, N_a = number of alleles over all loci. A_e^* = bias corrected effective number of alleles.

Native Region		Invasive Regions	
Locus and Allele Category	Allele Frequency in Region	Locus and Allele Category	Allele Frequency in Region
MV01-N1	0.132	MV01-I1	0.170
MV01-N2	0.107	MV01-I2	0.016
MV01-N3	0.223	MV03-I1	0.002
MV01-N4	0.058	MV03-I2	0.006
MV01-N5	0.041	MV03-I3	0.085
MV01-N6	0.004	MV03-I4	0.127
MV01-N7	0.021	MV03-I5	0.021
MV01-N8	0.066	MV09-I1	0.215
MV03-N1	0.351	MV05A-I1	0.703
MV03-N2	0.008	MV05A-I2	0.002
MV03-N3	0.128	MV05B-I1	0.039
MV03-N4	0.008	MV06-I1	0.696
MV10-N1	0.012	MV06-I2	0.018
MV10-N2	0.050	MV06-I3	0.063
MV10-N3	0.128	MV02-I1	0.022
MV09-N1	0.331	Average	0.146
MV09-N2	0.054		
MV09-N3	0.128		
MV09-N4	0.169		
MV05A-N1	0.068		
MV05A-N2	0.144	Total no. of private alleles in invasive region	15
MV05A-N3	0.059		
MV05A-N4	0.008		
MV05A-N5	0.161		
MV05A-N6	0.165		
MV05B-N1	0.059	Total no. of private alleles in native region	37
MV05B-N2	0.292		
MV06-N1	0.004		
MV06-N2	0.013		
MV06-N3	0.218		
MV06-N4	0.046		
MV07-N1	0.027		
MV07-N2	0.164		
MV08-N1	0.132		
MV08-N2	0.018		
MV08-N3	0.027		
MV08-N4	0.064		
Average	0.100		

Table 3.3. Summary of Private Alleles by Native (Asia) and Invasive (USA) Regions per locus

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	Est. Variance	Percent Variation	p-values	F-stat	F-stat value
Global								
Asia vs. US	1	348.18	348.18	0.75	22%	0.001	F_{RT}	0.22
Pops/Asia+US	32	1960.26	61.26	1.78	52%	0.001	F_{SR}	0.67
Individuals/Asia+US	536	923.01	1.72	0.84	25%	0.001	F_{IS}	0.95
Within Individuals	570	23.50	0.04	0.04	1%	0.001	F_{IT}	0.99
Native Region								
Among Regions/Asia	2	265.20	132.60	0.65	16%	0.001	F_{RT}	0.16
Among Pops+Region/Asia	7	335.79	47.97	2.24	55%	0.001	F_{SR}	0.66
Among Indiv./Asia	113	252.71	2.24	1.06	26%	0.001	F_{IS}	0.90
Within Individuals	123	14.00	0.11	0.11	3%	0.001	F_{IT}	0.97
Invasive Region								
Among Regions/US	2	925.93	462.96	2.24	63%	0.001	F_{RT}	0.63
Among Pops+Region/US	21	433.35	20.64	0.52	15%	0.001	F_{SR}	0.39
Among Indiv./US	423	670.30	1.59	0.78	22%	0.001	F_{IS}	0.97
Within Individuals	447	9.50	0.02	0.02	1%	0.001	F_{IT}	0.99

Table 3.4. Analysis of Molecular Variance (AMOVA) for the molecular dataset. This table represents three distinct AMOVAs (all samples, native samples only, and invasive samples only).

	Axis No.	1	2	3	4	5	6
PCO							
All samples		89.89	31.98	26.23	18.47	11.66	11.54
		47.37%	16.85%	13.82%	9.73%	6.15%	6.08%
Native (Asian) samples		28.46	26.51	22.93	11.45	10.85	8.79
		26.11%	24.33%	21.04%	10.51%	9.96%	8.06%
Invasive (USA) samples		86.05	34.87	24.92	9.30	6.51	5.32
		51.54%	20.88%	14.93%	5.57%	3.90%	3.19%

Table 3.5. Eigen values for the first six axes of the three PCO analyses performed with percent of each axis below the eigen value.

	CN11	CN12	CN13	CN14	CN15	CN16	CN17	CN3	CN7	J	AL	AR	CT	GA	IN	KN1	KN2	KN3	KN4	KN5	KN6	MD	MS1	MS2	NC	NJ	NY	OH	PA	SC1	SC2	VA1	VA2	WV	
CN11	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**CN11**
CN12	0.259	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**CN12**
CN13	0.710	0.920	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**CN13**
CN14	0.723	0.984	0.915	-	0.001	0.001	0.307	0.001	0.001	0.001	0.316	0.001	0.001	0.001	0.001	0.356	0.001	0.001	0.001	0.326	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.304	0.001	0.001	0.001	**CN14**
CN15	0.681	0.948	0.897	0.968	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**CN15**
CN16	0.477	0.693	0.534	0.688	0.644	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**CN16**
CN17	0.666	0.976	0.886	0.000	0.962	0.539	-	0.001	0.001	0.001	0.339	0.001	0.001	0.001	0.001	0.275	0.001	0.001	0.001	0.238	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.384	0.001	0.001	0.001	**CN17**
CN3	0.605	0.734	0.757	0.742	0.715	0.576	0.726	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**CN3**
CN7	0.561	0.684	0.694	0.676	0.645	0.488	0.663	0.266	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**CN7**
J	0.672	0.804	0.818	0.768	0.802	0.638	0.779	0.661	0.646	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**J**
AL	0.825	0.991	0.963	0.000	0.985	0.822	0.000	0.810	0.789	0.817	-	0.343	0.001	0.003	0.022	1.000	0.077	0.166	0.496	1.000	0.030	0.001	0.335	0.001	0.001	0.001	0.001	0.001	0.001	0.001	1.000	0.508	0.001	0.001	**AL**
AR	0.772	0.947	0.931	0.959	0.946	0.767	0.954	0.770	0.749	0.772	0.009	-	0.001	0.009	0.400	0.127	0.140	0.388	0.438	0.368	0.243	0.001	0.332	0.036	0.001	0.001	0.001	0.001	0.001	0.001	0.487	0.428	0.001	0.001	**AR**
CT	0.739	0.929	0.879	0.926	0.927	0.711	0.915	0.752	0.745	0.782	0.966	0.937	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**CT**
GA	0.738	0.930	0.916	0.946	0.932	0.732	0.938	0.740	0.719	0.744	0.151	0.068	0.926	-	0.041	0.192	0.406	0.023	0.013	0.079	0.392	0.001	0.018	0.062	0.003	0.001	0.001	0.001	0.001	0.001	0.013	0.011	0.001	0.001	**GA**
IN	0.716	0.864	0.877	0.879	0.874	0.703	0.866	0.718	0.701	0.707	0.037	0.000	0.886	0.035	-	0.377	0.343	0.444	0.383	0.406	0.249	0.001	0.339	0.405	0.001	0.001	0.001	0.001	0.001	0.001	0.145	0.311	0.001	0.001	**IN**
KN1	0.689	0.979	0.929	0.000	0.965	0.679	0.000	0.712	0.684	0.727	0.000	0.000	0.939	0.024	0.000	-	0.481	0.101	0.067	1.000	0.168	0.001	0.113	0.393	0.027	0.001	0.001	0.001	0.001	0.001	1.000	0.068	0.001	0.001	**KN1**
KN2	0.661	0.881	0.881	0.906	0.886	0.642	0.886	0.678	0.656	0.684	0.097	0.031	0.894	0.003	0.000	0.000	-	0.203	0.174	0.483	0.253	0.001	0.135	0.388	0.020	0.001	0.001	0.001	0.001	0.001	0.102	0.084	0.001	0.001	**KN2**
KN3	0.763	0.974	0.942	0.987	0.967	0.757	0.985	0.761	0.738	0.769	0.033	0.000	0.949	0.084	0.001	0.000	0.021	-	0.507	0.503	0.109	0.001	0.490	0.158	0.004	0.001	0.001	0.001	0.001	0.001	0.473	0.490	0.001	0.001	**KN3**
KN4	0.773	0.956	0.935	0.968	0.953	0.768	0.964	0.770	0.749	0.774	0.013	0.000	0.942	0.075	0.000	0.000	0.034	0.000	-	0.517	0.079	0.001	0.365	0.038	0.001	0.001	0.001	0.001	0.001	0.001	0.484	0.338	0.001	0.001	**KN4**
KN5	0.738	0.984	0.942	0.000	0.974	0.731	0.000	0.744	0.718	0.756	0.000	0.000	0.949	0.070	0.000	0.000	0.010	0.000	0.000	-	0.085	0.001	0.386	0.255	0.005	0.001	0.001	0.001	0.001	0.001	1.000	0.526	0.001	0.001	**KN5**
KN6	0.641	0.952	0.909	0.979	0.940	0.626	0.971	0.678	0.649	0.701	0.357	0.000	0.922	0.000	0.000	0.073	0.000	0.089	0.019	0.170	-	0.001	0.323	0.300	0.100	0.001	0.001	0.001	0.001	0.001	0.058	0.054	0.001	0.001	**KN6**
MD	0.556	0.669	0.699	0.701	0.712	0.519	0.579	0.585	0.563	0.600	0.702	0.657	0.681	0.620	0.612	0.584	0.559	0.645	0.656	0.622	0.544	-	0.001	0.001	0.001	0.001	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.003	**MD**
MS1	0.781	0.957	0.937	0.969	0.955	0.775	0.965	0.775	0.754	0.780	0.009	0.000	0.943	0.065	0.005	0.000	0.030	0.000	0.000	0.000	0.000	0.661	-	0.041	0.001	0.001	0.001	0.001	0.001	0.001	0.490	0.339	0.001	0.001	**MS1**
MS2	0.684	0.810	0.839	0.829	0.831	0.669	0.807	0.692	0.675	0.670	0.062	0.037	0.850	0.030	0.000	0.000	0.000	0.018	0.036	0.011	0.000	0.581	0.038	-	0.002	0.001	0.001	0.001	0.001	0.001	0.035	0.016	0.001	0.001	**MS2**
NC	0.530	0.635	0.682	0.621	0.652	0.481	0.597	0.565	0.537	0.503	0.250	0.203	0.679	0.185	0.156	0.128	0.115	0.191	0.200	0.170	0.098	0.429	0.210	0.133	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**NC**
NJ	0.489	0.655	0.613	0.640	0.668	0.435	0.596	0.507	0.514	0.539	0.745	0.699	0.398	0.664	0.653	0.616	0.594	0.683	0.698	0.658	0.575	0.476	0.703	0.624	0.467	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**NJ**
NY	0.729	0.898	0.833	0.897	0.900	0.685	0.869	0.753	0.742	0.770	0.941	0.914	0.456	0.901	0.870	0.905	0.871	0.920	0.916	0.917	0.889	0.686	0.919	0.839	0.688	0.319	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**NY**
OH	0.803	0.963	0.937	0.973	0.960	0.794	0.969	0.750	0.737	0.805	0.974	0.934	0.937	0.919	0.859	0.956	0.885	0.956	0.942	0.962	0.938	0.700	0.943	0.807	0.658	0.574	0.909	-	0.001	0.001	0.001	0.001	0.001	0.001	**OH**
PA	0.659	0.777	0.791	0.807	0.807	0.621	0.721	0.667	0.649	0.684	0.822	0.782	0.799	0.754	0.731	0.737	0.702	0.779	0.783	0.764	0.706	0.092	0.788	0.698	0.549	0.603	0.792	0.811	-	0.001	0.001	0.001	0.001	0.001	**PA**
SC1	0.696	0.867	0.870	0.866	0.864	0.698	0.868	0.735	0.712	0.733	0.526	0.417	0.879	0.421	0.333	0.389	0.351	0.447	0.422	0.432	0.352	0.645	0.435	0.325	0.273	0.637	0.863	0.866	0.758	-	0.001	0.001	0.001	0.001	**SC1**
SC2	0.793	0.989	0.955	0.000	0.981	0.789	0.000	0.784	0.762	0.793	0.000	0.000	0.960	0.119	0.020	0.000	0.062	0.013	0.000	0.000	0.284	0.670	0.000	0.043	0.218	0.711	0.932	0.969	0.799	0.488	-	0.516	0.001	0.001	**SC2**
VA1	0.792	0.960	0.941	0.971	0.958	0.787	0.968	0.784	0.764	0.787	0.004	0.000	0.946	0.084	0.004	0.000	0.045	0.000	0.000	0.000	0.034	0.673	0.000	0.043	0.217	0.716	0.922	0.946	0.796	0.445	0.000	-	0.001	0.001	**VA1**
VA2	0.550	0.675	0.666	0.665	0.689	0.492	0.614	0.579	0.545	0.572	0.742	0.703	0.682	0.673	0.662	0.637	0.615	0.691	0.701	0.670	0.603	0.195	0.707	0.641	0.500	0.476	0.676	0.700	0.188	0.674	0.714	0.717	-	0.001	**VA2**
WV	0.430	0.531	0.576	0.552	0.583	0.378	0.446	0.432	0.436	0.427	0.560	0.511	0.478	0.464	0.463	0.409	0.385	0.488	0.508	0.457	0.360	0.135	0.514	0.429	0.281	0.219	0.500	0.419	0.266	0.517	0.519	0.530	0.256	-	**WV**
	CN11	**CN12**	**CN13**	**CN14**	**CN15**	**CN16**	**CN17**	**CN3**	**CN7**	**J**	**AL**	**AR**	**CT**	**GA**	**IN**	**KN1**	**KN2**	**KN3**	**KN4**	**KN5**	**KN6**	**MD**	**MS1**	**MS2**	**NC**	**NJ**	**NY**	**OH**	**PA**	**SC1**	**SC2**	**VA1**	**VA2**	**WV**	

Table 3.6. Matrix of pairwise population F_{ST}-values below diagonal. P-value in support of each F_{ST}-value above diagonal. Shaded values indicate insignificant relationships (P>0.01).

	CN11	CN12	CN13	CN14	CN15	CN16	CN17	CN3	CN7	J	AL	AR	CT	GA	IN	KN1	KN2	KN3	KN4	KN5	KN6	MD	MS1	MS2	NC	NJ	NY	OH	PA	SC1	SC2	VA1	VA2	WV	
CN11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN11**
CN12	0.024	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN12**
CN13	0.702	0.801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN13**
CN14	0.644	0.629	0.474	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN14**
CN15	0.623	0.629	0.626	0.626	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN15**
CN16	0.579	0.664	0.197	0.520	0.520	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN16**
CN17	0.610	0.476	0.345	0.626	0.801	0.268	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN17**
CN3	0.787	0.640	0.801	0.621	0.589	0.674	0.639	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN3**
CN7	0.632	0.546	0.606	0.474	0.456	0.476	0.541	0.072	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CN7**
J	0.737	0.606	0.778	0.338	0.630	0.585	0.463	0.468	0.527	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**J**
AL	0.608	0.461	0.801	0.474	0.626	0.541	0.474	0.483	0.545	0.239	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**AL**
AR	0.607	0.461	0.801	0.474	0.626	0.541	0.474	0.483	0.545	0.239	0.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**AR**
CT	0.748	0.801	0.474	0.474	1.000	0.563	0.474	0.626	0.801	0.435	0.801	0.801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**CT**
GA	0.603	0.461	0.777	0.474	0.629	0.525	0.474	0.483	0.545	0.235	0.001	0.001	0.801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**GA**
IN	0.607	0.461	0.801	0.474	0.626	0.541	0.474	0.483	0.544	0.239	0.000	0.000	0.801	0.001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**IN**
KN1	0.604	0.461	0.801	0.474	0.626	0.538	0.474	0.482	0.544	0.237	0.000	0.000	0.801	0.001	0.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**KN1**
KN2	0.605	0.461	0.801	0.474	0.627	0.530	0.474	0.483	0.544	0.234	0.000	0.000	0.801	0.000	0.000	0.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**KN2**
KN3	0.606	0.461	0.801	0.474	0.626	0.541	0.474	0.483	0.545	0.238	0.000	0.000	0.801	0.001	0.000	0.000	0.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**KN3**
KN4	0.607	0.461	0.801	0.474	0.626	0.541	0.474	0.483	0.545	0.238	0.000	0.000	0.801	0.001	0.000	0.000	0.000	0.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**KN4**
KN5	0.606	0.461	0.801	0.474	0.626	0.540	0.474	0.483	0.544	0.238	0.000	0.000	0.801	0.001	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**KN5**
KN6	0.602	0.461	0.801	0.474	0.626	0.536	0.474	0.481	0.543	0.236	0.000	0.000	0.801	0.001	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**KN6**
MD	0.000	0.613	0.773	0.000	0.975	0.601	0.345	0.591	0.640	0.424	0.323	0.322	0.597	0.322	0.320	0.319	0.321	0.319	0.322	0.321	0.317	-	-	-	-	-	-	-	-	-	-	-	-	-	**MD**
MS1	0.607	0.461	0.801	0.474	0.626	0.541	0.474	0.483	0.545	0.239	0.000	0.000	0.801	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.322	-	-	-	-	-	-	-	-	-	-	-	-	**MS1**
MS2	0.587	0.450	0.776	0.465	0.620	0.515	0.462	0.484	0.540	0.236	0.001	0.001	0.776	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.311	0.001	-	-	-	-	-	-	-	-	-	-	-	**MS2**
NC	0.577	0.480	0.714	0.429	0.615	0.479	0.440	0.518	0.535	0.238	0.067	0.067	0.639	0.067	0.067	0.063	0.066	0.065	0.067	0.065	0.060	0.273	0.067	0.055	-	-	-	-	-	-	-	-	-	-	**NC**
NJ	0.613	0.769	0.490	0.607	0.903	0.437	0.577	0.480	0.615	0.411	0.561	0.560	0.144	0.560	0.560	0.558	0.559	0.560	0.560	0.559	0.556	0.528	0.560	0.533	0.459	-	-	-	-	-	-	-	-	-	**NJ**
NY	0.748	0.801	0.345	0.626	1.000	0.452	0.474	0.626	0.801	0.446	0.801	0.801	0.009	0.801	0.801	0.801	0.801	0.801	0.801	0.801	0.801	0.610	0.801	0.776	0.674	0.164	-	-	-	-	-	-	-	-	**NY**
OH	0.782	0.611	0.801	0.626	0.801	0.687	0.626	0.356	0.456	0.336	0.237	0.237	0.626	0.237	0.237	0.237	0.237	0.237	0.237	0.237	0.237	0.499	0.237	0.236	0.305	0.277	0.626	-	-	-	-	-	-	-	**OH**
PA	0.711	0.601	0.738	0.731	0.920	0.598	0.372	0.580	0.642	0.421	0.358	0.357	0.678	0.357	0.356	0.355	0.356	0.354	0.357	0.356	0.353	0.032	0.357	0.347	0.318	0.584	0.678	0.504	-	-	-	-	-	-	**PA**
SC1	0.482	0.601	0.801	0.474	0.624	0.541	0.616	0.611	0.620	0.335	0.008	0.008	0.801	0.010	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.430	0.008	0.009	0.046	0.487	0.801	0.337	0.477	-	-	-	-	-	**SC1**
SC2	0.607	0.461	0.801	0.474	0.626	0.541	0.474	0.483	0.545	0.238	0.000	0.000	0.801	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.322	0.000	0.001	0.067	0.560	0.801	0.237	0.357	0.008	-	-	-	-	**SC2**
VA1	0.607	0.461	0.801	0.474	0.626	0.541	0.474	0.483	0.545	0.239	0.000	0.000	0.801	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.323	0.000	0.000	0.067	0.560	0.801	0.237	0.358	0.008	0.000	-	-	-	**VA1**
VA2	0.797	0.734	0.665	0.635	0.910	0.552	0.509	0.603	0.580	0.378	0.534	0.533	0.674	0.533	0.531	0.529	0.531	0.531	0.533	0.532	0.527	0.106	0.533	0.524	0.436	0.522	0.634	0.477	0.077	0.630	0.533	0.533	-	-	**VA2**
WV	0.682	0.606	0.729	0.650	0.888	0.547	0.431	0.476	0.560	0.334	0.315	0.315	0.414	0.314	0.314	0.309	0.312	0.311	0.315	0.312	0.305	0.102	0.315	0.292	0.201	0.189	0.422	0.253	0.163	0.415	0.315	0.315	0.263	-	**WV**
	CN11	**CN12**	**CN13**	**CN14**	**CN15**	**CN16**	**CN17**	**CN3**	**CN7**	**J**	**AL**	**AR**	**CT**	**GA**	**IN**	**KN1**	**KN2**	**KN3**	**KN4**	**KN5**	**KN6**	**MD**	**MS1**	**MS2**	**NC**	**NJ**	**NY**	**OH**	**PA**	**SC1**	**SC2**	**VA1**	**VA2**	**WV**	

Table 3.7. Pairwise populations matrix of harmonic means of Jost's D_{est} of genetic differentiation between populations. Shaded values indicate $D_{est}<0.002$.

Chapter 4

Evidence for rapid adaptive evolution of phenology across the invasive range of *Microstegium vimineum*

Abstract

Evolutionary dynamics of integrative traits such as reproductive phenology are predicted to be critically important to invasion success, yet there are relatively few empirical examples evaluating the importance of such phenomena for invasive species. In this study, I used a multiple common garden approach to examine the evolutionary significance of latitudinal variation in phenology in a successful biological invader, the Asian short-day flowering annual grass *Microstegium vimineum*. I grew plants from seeds collected from multiple latitudes across the species' invasive range in environmentally controlled growth chambers to quantify phenological patterns that may have arisen via evolutionary processes. I observed that flowering time and biomass were both strongly correlated with the latitude of population origin such that populations collected from more northern latitudes flowered significantly earlier, at lower biomass, than populations from southern locations. I argue that this pattern must be the result of rapid adaptive evolution of phenology over a period of less than one hundred years, and such changes have likely promoted the northward range expansion of this species. I note that possible barriers to gene flow, including bottlenecks and

inbreeding, have apparently not forestalled adaptive processes for this plant. Furthermore, I conjecture that adaptive evolution of phenology may be widespread in many invasive plant species and an essential process during range expansion.

Introduction

Biological invasions may be enabled by biotic or abiotic characteristics of invaded habitats, traits of the introduced species, or some combination (Catford et al. 2009, Gurevitch et al. 2011). Theoretical and empirical studies suggest that evolution during the invasion process may be an important but underappreciated facet of biological invasions (Baker 1974, Lee 2002, Novak 2007, Lankau et al. 2009, Dormontt et al. 2011). Colonization by any species of novel habitats generally results in exposure to novel selective regimes (Suarez and Tsutsui 2008), founder effects, genetic drift, and/or hybridization events (Bossdorf et al. 2005). Rapid evolution may be a key feature of range expansions for any species (Maron et al. 2004, Montague, Barrett and Eckert 2007, Xu et al. 2010). Evolution may even be antecedent to a new arrival becoming invasive, perhaps explaining the lag time that sometimes occurs during the invasion process (Crooks 2005). Despite the often-cited possibility that invasions are enabled by evolutionary processes, there are relatively few empirical studies examining the phenomenon (Colautti, Maron and Spencer 2009).

Studies of life history evolution (e.g., Griffith and Watson 2006) and community ecology theory (e.g., Wolkovich and Cleland 2011) have both been leveraged to suggest that evolution of phenology (i.e., the seasonal timing of reproduction and other life history events) ought to be an important aspect of range expansion and invasion success. Phenology has been shown to respond to various selective pressures (Griffith and Watson 2006, Franks et al. 2007). In

particular, genetically controlled phenological timing has been associated with fitness benefits through interaction with frost avoidance (Kuser and Ching 1980), climate change (Bradshaw and Holzapfel 2001), growth rates (Blair and Wolf 2004), defense responses (Meyer and Hull-Sanders 2008), reproductive rates (Brown and Eckert 2005), plasticity (Lavergne and Molofsky 2007), and trade-offs with size at reproduction (Colautti, Eckert and Barrett 2010). Furthermore, the quantitative genetic nature of flowering timing in plants (Chardon et al. 2004) increases the likelihood of observing evolution of phenology in nature since quantitative traits present more genetic targets for selection.

In this study, I evaluated the role of rapid evolutionary processes as a widespread enabler of biological invasions by examining patterns of variation in two key life-history traits, reproductive phenology and size at reproduction, in the invasive grass *Microstegium vimineum* in eastern North America. I interpret my findings as evidence of rapid adaptive evolution of a life-history tradeoff between these two traits in this invasive plant. I further posit that the dearth in genetic diversity due to founder effects, which probably accompanied *M. vimineum* invasion, did not prove sufficient to forestall adaptive evolution in this species and that evolution of phenology may be a common process associated with plant invasions.

Materials and Methods

Study species

Microstegium vimineum (Trin.) A. Camus (Japanese stiltgrass, family Poaceae, order Poales) is a C4 annual grass native to Asia, where it is found in various habitats, including forest margins and riparian areas (Chen & Phillips 2007). It was first recorded in 1919 in Tennessee (Fairbrothers & Gray 1972), is now invasive in more than 20 U.S. states (USDA and NRCS 2005), and continues to spread rapidly. *Microstegium vimineum* flowers in the fall in response to short days (Judge 2006) and produces abundant seed that is dispersed by water, animals, recreational activities, mowing and timber harvests. Invasive *M. vimineum* is often first found along roads, trails, and streams, but it can also colonize full sun forest openings, shaded forests, and riparian areas (Cheplick 2010; Flory 2010), where it forms dense and persistent populations. In spite of being a C4 grass, it is highly shade tolerant and can produce seed under very low-light conditions (Cheplick 2010; Horton & Neufeld 1998). The abundance of *M. vimineum* in the field is highly correlated with light availability (Cheplick 2010; Flory 2010), however, it grows best under high light and high moisture conditions in experimental microcosms (Droste et al. 2010).

Collection of plant material

I collected *M. vimineum* seeds from 10 U.S. populations, representing the majority of latitudinal variation in its invasive range (Table 4.1, Figure 4.1). For each population, many hundreds to thousands of seeds were randomly collected from many individual stems, mostly from terminal inflorescences, within a circular area of 20 m diameter. Seeds were air dried and stored in paper bags at room temperature until sowing.

Experimental design

To quantify genetic variation in phenology among invasive *M. vimineum* populations, I grew all populations under common garden conditions in growth chambers. To evaluate phenological responses generally, as opposed to under one specific latitudinal habitat, I manipulated day length in four 8.9 m^2 controlled growth chambers (model GC-96-11-CW-C3, Environmental Growth Chambers Inc., Chagrin Falls, OH). Two growth chambers were set to simulate growing season daylength conditions at the northern extreme of *M. vimineum's* invasive range, approximately 42° N latitude, while the other two chambers simulated growing season conditions at the southern extreme, approximately 34° N latitude (USDA and NRCS 2005). The growth chambers were set to simulate light conditions beginning on June 1 and progressing as under natural conditions for the duration of the experiment (see Appendix Table B.2). Daylength progressions

were determined using U.S. Naval Observatory tables (http://aa.usno.navy.mil/). Humidity was set to a constant 70% and temperatures were set to 26 and 22°C for day and night, respectively, in all growth chambers.

I germinated randomly selected seeds from each population in individual four inch plastic pots, one plant per pot, filled with Fafard Growing Mix 2 (Conrad Fafard Inc., Agawam, MA). Pots were then randomized into five blocks in each chamber to control for within chamber environmental heterogeneity, and separated by at least 5 cm to prevent plants from rooting in neighboring pots and to minimize light competition among plants. Each chamber contained five randomized blocks with two plants from each of the ten populations in each block, for a total of 100 plants (each in its own pot) per chamber and a total of 400 plants across all four chambers (see Appendix Table B.3). Plants were watered every other day and were fertilized with dilute 20-20-20 NPK liquid fertilizer (Scotts Co., Maysville, OH) and iron chelate (Becker Underwood, Inc., Ames, IA) bi-weekly.

Data collection

I visually inspected all plants daily for signs of flowering. I recorded the date of first anthesis for each plant, and tabulated the number of days from germination to anthesis. Plants were allowed to grow until senescence, defined as all flowering complete, terminal seeds fully mature, and with less than 30%

(visual assessment) of the plant green. As each plant reached senescence, it was removed from the growth chamber. Above and belowground biomass were separated, roots were washed to remove soil, and all biomass was dried at 60°C to constant mass and weighed. Above and belowground mass were determined separately and summed to calculate total plant biomass.

Data analysis

To determine whether latitude of *M. vimineum* origin determined phenology, I analyzed days to anthesis and plant biomass, using mixed model analyses of variance (ANOVAs; Proc Mixed, SAS Institute, 2008). Population origin, north/south light treatment, and their interaction were modeled as fixed effects, and chamber, block, and their interaction were modeled as random effects. Significant effects of population origin on flowering time or biomass measurements of *M. vimineum* would indicate genetic determination and likely local adaptation of these traits. Genetic effects are likely to be relatively uniform within *M. vimineum* populations, due to high rates of inbreeding that result from self-compatibility and a large proportion of the flowers being cleistogamous (i.e., obligately inbreeding, see Chapter 3). Cheplick (2007) found that *M. vimineum* biomass allocation to cleistogamous flowers was over twice that of allocation to chasmogamous flowers in edge habitats and approximately 15% higher in shaded habitats. Even the terminal, chasmogamous inflorescences are likely to

promote inbreeding, due to the plant's receptivity to self-pollen (which is present when stigmas have exerted). Furthermore, results from Chapter 3 demonstrate that inbreeding is leading to near allelic fixation and, hence, genetically uniform populations. I also performed regression analyses (SigmaPlot 11.0, Systat Software, Inc., 2008) to compare days to anthesis and biomass responses to the latitude of source populations.

Results

Of the 400 individuals planted in the growth chambers, 373 were included in the dataset for biomass. The 27 plants not included had suffered mechanical injuries during the course of the experiment that interfered with normal growth progression and, therefore, final biomass. The damage occurred before anthesis in only two of these cases, and those two plants were not included in the dataset for days to anthesis.

Overall, I observed a clear cline in both time to anthesis (Table 4.1 and Figure 4.2) and biomass (Figure 4.3), based on latitudinal origin of populations, with more northern populations flowering earlier and producing less biomass. Southern populations reached anthesis later than did populations from farther north, under both northern and southern photoperiods. All populations reached anthesis later under northern photoperiods than they did under southern photoperiods (Table 4.1).

Days to anthesis were negatively correlated with latitudinal population origin for both the northern (r^2 = 0.847; P < 0.001; Fig. 4.2A) and southern (r^2 = 0.835; P < 0.001; Fig. 4.2B) light treatments. Under the northern light treatment, the average time to anthesis ranged from 79.0 days to 119.5 days, corresponding to a critical photoperiod at anthesis of between 10h:54min to 12h:56min, for northern and southern sourced plants respectively. For the southern light treatment, average days to anthesis among the populations was 70.1 to 105.7, corresponding to a critical photoperiod at anthesis of 11h:42min to 12h:48min, for northern and southern sourced plants respectively.

Similar patterns were exhibited in biomass responses for both light treatments with biomass also negatively correlated with latitudinal population origin. For the northern light treatment, mean root biomass ranged from 0.997 to 2.180 g (r^2 = 0.748, P < 0.001; Fig. 4.3A), aboveground biomass ranged from 3.485 to 5.870 g (r^2 = 0.633, P = 0.006; Fig. 4.3C), and total biomass ranged from 4.482 to 7.882 g (r^2 = 0.633, P = 0.002; Fig. 4.3E), with a clear north-south gradient. For the southern light treatment, mean root biomass ranged from 0.734 to 1.690 g (r^2 = 0.712, P = 0.002; Fig. 4.3B), aboveground biomass ranged from 2.815 to 4.715 g (r^2 = 0.604, P = 0.008; Fig. 4.3D), and total biomass ranged from 3.711 to 6.370 g (r^2 = 0.704, P = 0.002; Fig. 4.3F), with a clear north-south gradient. There were significant effects of both population origin and the north/south light treatment on time to anthesis and all biomass measurements. However, only days to anthesis and root biomass exhibited significant

interactions between population origin and light treatment. With the exception of the chamber effects on days to anthesis, I found no significant random effects of chamber, block, or their interactions (Table 4.2). In summary, plants from higher latitudes flowered earlier and produced less biomass than plants from more southern populations.

Discussion

Adaptive evolution of phenology

These results clearly demonstrate a strong latitudinal cline for the number of days required to reach anthesis and the amount of biomass produced by *M. vimineum* populations collected from throughout its invasive range. Growing plants in a common environment in growth chambers allowed us to demonstrate that these traits are most likely under strong genetic control, while replication of the experiment under two distinct light regimes confirmed that these trends are generalized findings, independent of specific local light regimes. Absent maternal effects, which are yet to be observed and unlikely for such plant life history traits (Montague, Barrett and Eckert 2007), these observed population differences clearly indicate divergent phenological and biomass allocation characters under genetic control. Moreover, clinal variation in the traits is most likely due to adaptive evolution, as a result of selective pressure to complete flowering and seed maturity before the end of the growing season (i.e., cold temperatures

arresting seed maturity) at more northern latitudes. The results suggest that such evolution of phenological traits has permitted the rapid expansion of *M. vimineum* invasions into more northern habitats.

Although I did not measure fitness consequences of flowering time and biomass variation directly in this study, adaptive evolution of phenological timing is the most likely explanation for what I observed during this experiment. The only other possible explanation would be that native *M. vimineum* propagules were transported from latitudes in Asia directly to equivalent latitudes in North America. This occurrence would represent pre-adaptation but is highly unlikely, given the available herbarium records in North America. The plant was first noticed in the southeastern United States by the 1910s, and then radiated northward and westward (Fairbrothers and Gray 1972). Though I cannot preclude the possibility of multiple introductions (which are probably likely), even such introductions would almost certainly have been discrete events, located at major shipping locations, as the plant has been reported to be introduced as packing material for ceramics imported to North America from central China (Dorman 2008). Furthermore, steady range expansion of *M. vimineum* across North America, particularly northward, has been noted in recent years (Mehrhoff 2002). Assuming that Dorman (2008) is correct in asserting that the species was introduced from the ceramics regions of central China (mainly in Janxi Province), the initial invasive propagules of *M. vimineum* would have derived from regions below 31° N latitude. This would imply that genetic determination of phenology in

the invasive range samples included in this study have evolved *in situ* since they were all collected north of 33° N latitude.

Most interestingly, the adaptive evolutionary patterns I observed here must have arisen over a 100 year period or less. I am aware of three other genera of invasive plants for which similar phenological clines have developed after initial colonization, *Lythrum salicaria* in North America (Montague, Barrett and Eckert 2007), two *Solidago* species in Europe (Weber and Schmid 1998) and *Impatiens glandulifera* in Europe (Kollmann and Bañuelos 2004). Interestingly, both the invasive *Solidago* species and *L. salicaria* are self-incompatible, while *I. glandulifera* is self-compatible but protandrous to promote outcrossing. Therefore, *M. vimineum* is the first invasive plant species identified that has evolved clinal phenological variation in its invasive range, but does not possess biology favoring, or requiring, outcrossing. In fact, *M. vimineum*'s biology promotes inbreeding due to cleistogamy. *Microstegium vimineum* also seems to have evolved clinal phenology in a shorter period of time than these other species, which were all introduced in their invasive ranges by the early 1800's (Weber and Schmid 1998, Blossey, Skinner and Taylor 2001, Kollmann and Bañuelos 2004). However it should be noted that the time between introduction and discovery of clinal phenological patterns represents a maximum time of phenological evolution. Evolution for any of these species may have occurred over a much shorter period and simply evaded our notice.

Microstegium vimineum seems to have undergone a lag phase from the time it was first recorded in the early 1900s until it was recognized as an invasive species in the late 1980s (Barden 1987). As a fecund annual with a relatively short-lived seed bank, *M. vimineum* possesses the potential for rapid adaptation, given adequate genetic diversity. At the minimum, it has cycled through approximately 100 generations in the invasive range, though adaptive evolution is likely to have taken place over a much shorter period of time in areas where the plant has only existed for a few decades (e.g., New England). Apparently, a tendency to inbreed has not impaired this species' ability to evolve clinal phenology, as it has possibly done so even more rapidly than the outbreeding species that have evolved similar patterns. Interestingly, I observed a general trend toward smaller variance in days to anthesis for populations from the extremes of the invasive range, compared with the center of the range (Fig. 4.2A,B). This could be a result of limited genetic diversity at the edges of the range, due to decreased gene flow or stronger selection under the more extreme climate regimes expected at the northernmost range extents.

Biomass

Both above and belowground *M. vimineum* biomass decreased with increasing latitudinal origin of populations. Because *M. vimineum* biomass is strongly correlated with seed production (total chasmogamous and cleistogamous seeds, $r^2 = 0.90$, $n = 24$, S.L. Flory, *personal communication*),

reduced biomass from more northern populations probably indicates decreased seed production, relative to more southern populations, which was also found for invasive populations of *L. salicaria* (Colautti, Eckert and Barrett 2010). It has long been appreciated that for short day flowering plants, local survival of a plant species depends on the production of viable seeds before frost (or other inhospitable climate conditions) arrests metabolism (e.g., Allard 1932). Since the optimal flowering time, where reproductive output is maximized before seasonal climatic conditions become unfavorable, will vary with photoperiod latitudinally, short day flowering plants can be expected to evolve appropriate critical photoperiods for each local habitat, thus maximizing reproductive success. For *M. vimineum*, this has resulted in evolution of a life-history tradeoff between flowering time and size at reproduction.

Potential Genetic Mechanisms

Though the flowering time pathways of higher plants have mostly been elucidated in the model species *Arabidopsis thaliana*, which flowers under long day conditions, studies of rice (*Oryza sativa*) have revealed many of the genetic and molecular details associated with short day flowering (e.g., Hayama and Coupland 2004). Rice cultivars exhibit a latitudinal cline in flowering time well north of the range limit of ancestral wild rice (by approximately 14° latitude), most likely as a result of artificial selection by farmers over thousands of years. At

northern latitudes, the available climatic window for flower formation, meiosis in pollen development and embryogenesis leading to edible seeds, is limited by the earlier onset of cold weather conditions (Izawa 2007). Though research has not yet elucidated all of the molecular mechanisms explaining the continuous distribution of flowering time phenotypes in rice, it is clear that there are multiple genetic elements controlling floral pathways that can be considered quantitative flowering time traits. Quantitative traits would present a multitude of genetic elements that could be selected for and may therefore be particularly amenable to rapid evolutionary processes. Furthermore, there is significant homology between rice and *Arabidopsis* flowering genes, including floral promoters and the florigen (FT) genes (Yano et al. 2001, Izawa 2007). Since *M. vimineum* is a short day flowering plant in the grass family, and flowering genes have been shown to be highly conserved across the plant kingdom, *M. vimineum* is likely to possess flowering control mechanisms similar to those observed in con-familial rice.

Interestingly, high rates of genetic diversity would not necessarily be required to evolve diverse phenological phenotypes. As a highly quantitative trait, even few alleles, acting epistatically among many genes, would result in the potential to express widely divergent, even continuous, phenotypes. The clinal variation I observed could result from either the rapid evolution of new alleles in the flowering timing pathways that developed post-invasion, or they might have resulted from selection on existing alleles from the native range that survived the transfer to North America. As a highly quantitative trait, generation of new alleles

in the invasive range may not have been necessary to drive evolution in this case, but still may have occurred.

I have also conducted microsatellite (SSR) marker analysis on over thirty populations of *M. vimineum* from its native and invasive ranges (see Chapter 3). I found that genetic diversity, measured both by heterozygosity and effective number of alleles, was lower in the invasive range, a clear indication of at least some degree of founder effect. Despite an initial bottleneck and high levels of inbreeding, *M. vimineum* has been able to evolve adaptive clinal variation in phenology over approximately 100 generations.

Conclusions

These results demonstrate rapid evolution of phenology in the highly invasive grass *M. vimineum,* whereby flowering time and biomass allocation are strongly correlated with the latitude of population origin. I hypothesize that adaptive evolution via selection on flowering time is implicated, at least in part, for the northward spread of this species in the eastern United States. Moreover, *M. vimineum* is a non-clonal, inbreeding, annual grass. The few other invasive plant species that have demonstrated a similar pattern of clinal evolution in phenology include clonal, obligately outcrossing, and perennial species in widely divergent families and orders (Lythraceae, Myrtales; Balsaminaceae, Ericales; Asteraceae, Asterales), suggesting that rapid adaptive evolution of phenology

may be widespread and critically important in the range expansion or invasion of many plant species, despite potential limitations to gene flow and probable historical bottlenecks.

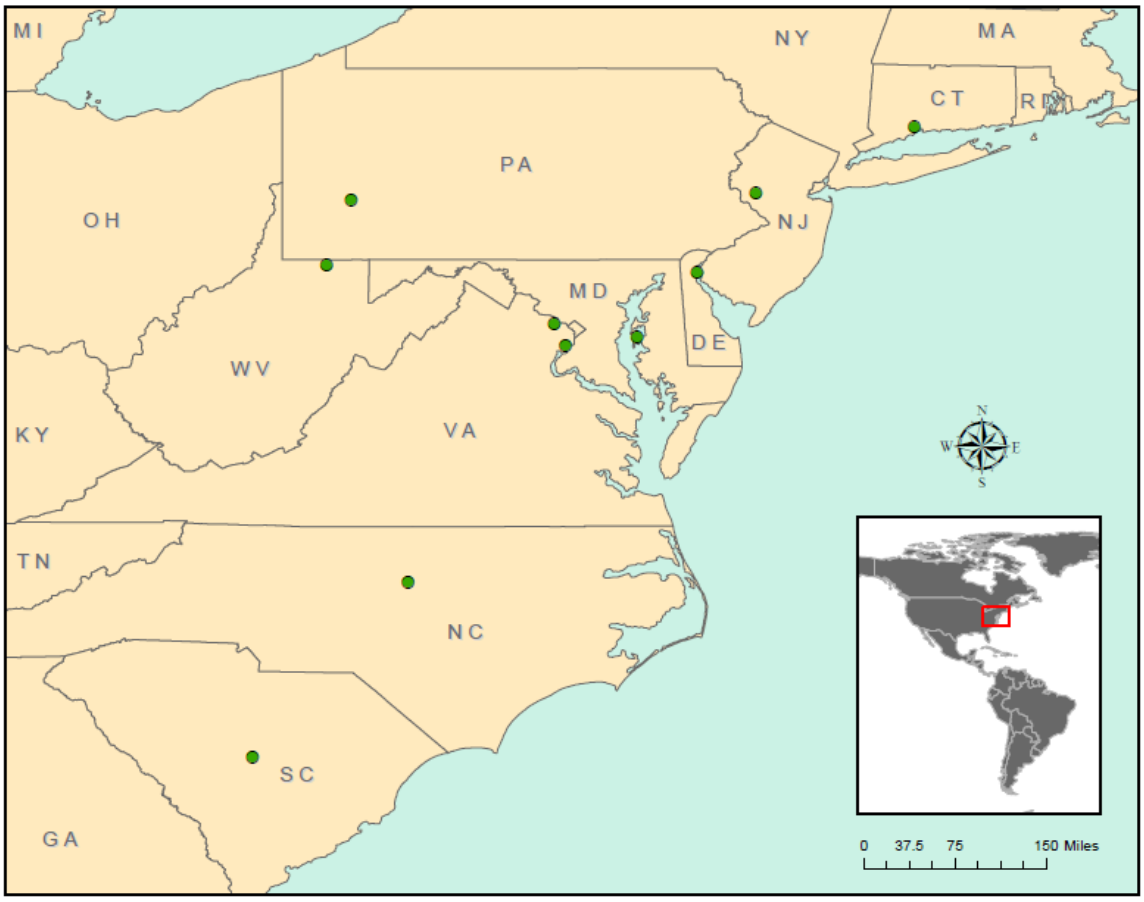


Figure 4.1. Location of seed collection in the eastern USA for *M. vimineum* plants used in the growth chamber study.

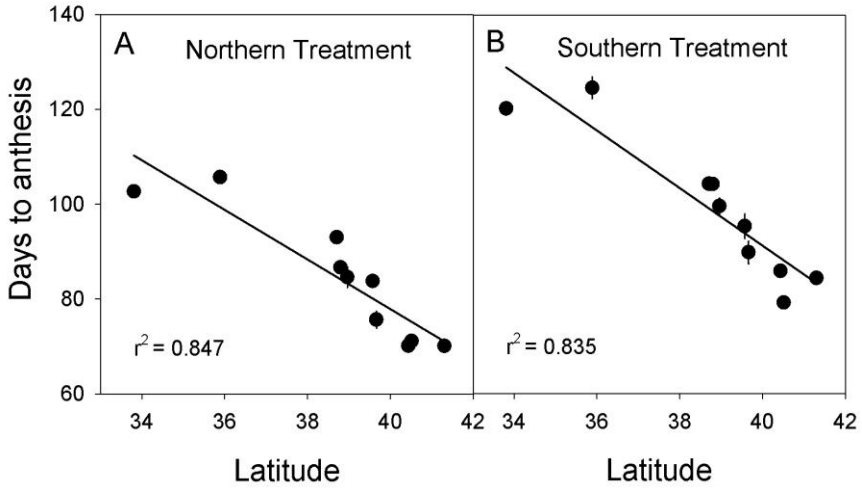


Figure 4.2. Relationship between latitude of population origin and days to anthesis of *M. vimineum* under the northern (A) and southern (B) light treatments. Bars indicate standard errors.

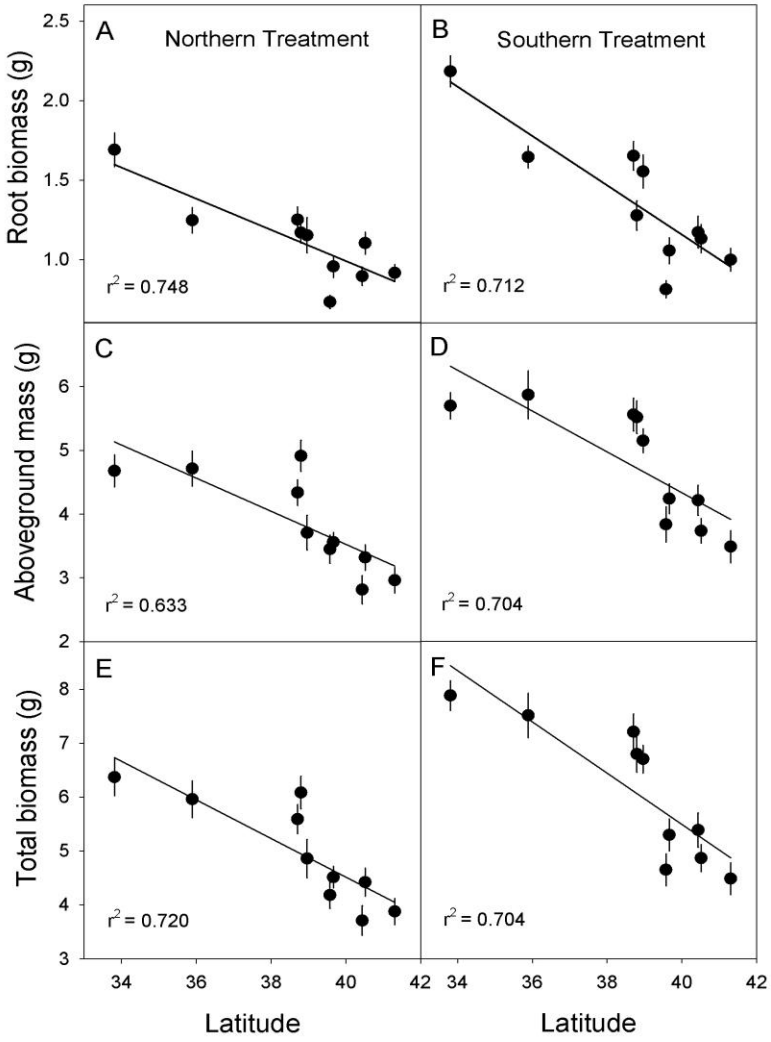


Figure 4.3. Relationship between latitude of population origin and *M. vimineum* performance under the northern and southern light treatments as measured by root biomass (A,B), aboveground biomass (C,D), and total plant biomass (E,F). Bars indicate standard errors.

					Mean Days to Anthesis (± SE)	
Pop	**State**	**Nearest town**	**Latitude**	**Longitude**	**Northern**	**Southern**
1	South Carolina	Hopkins	33° 48' 28" N	80° 51' 55" W	119.5 (±1.4)	102.7 (±0.4)
2	North Carolina	Chapel Hill	35° 53' 24" N	79° 00' 56" W	123.8 (±2.3)	105.7 (±0.9)
3	Virginia	Fort Belvoir	38° 42' 22" N	77° 08' 48" W	103.8 (±1.2)	93.1 (±0.5)
4	Maryland	Whittman	38° 47' 43" N	76° 17' 40" W	103.7 (±0.6)	86.7 (±0.5)
5	Virginia	Great Falls	38° 57' 44" N	77° 16' 44" W	99.1 (±1.7)	84.6 (±1.2)
6	Delaware	Delaware City	39° 34' 22" N	75° 34' 50" W	94.9 (±2.5)	83.8 (±1.1)
7	West Virginia	Morgantown	39° 39' 45" N	79° 59' 00" W	89.5 (±2.3)	75.6 (±1.8)
8	Pennsylvania	Murraysville	40° 26' 05" N	79° 41' 50" W	85.6 (±0.9)	70.1 (±0.5)
9	New Jersey	Flemington	40° 30' 44" N	74° 53' 04" W	79.0 (±0.7)	71.1 (±0.5)
10	Connecticut	Orange	41° 18' 18" N	72° 59' 54" W	84.1 (±1.0)	70.1 (±0.7)

Table 4.1. Collection locations in the United States for the 10 invasive *M. vimineum* populations sampled and their mean time to anthesis under the northern and southern light treatments.

Fixed effects										
Source of variation	Num d.f.	Den d.f	Days to anthesis		Total biomass (g)		Aboveground biomass (g)		Root biomass (g)	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Population	9	373	210.23	**<.0001**	26.65	**<.0001**	23.05	**<.0001**	32.94	**<.0001**
Treatment	1	373	65.38	**<.0001**	66.29	**<.0001**	45.14	**<.0001**	4.64	**0.03**
Pop. x Treat.	9	373	2.83	**<.0001**	1.76	0.07	1.44	0.16	2.31	**0.02**
Random effects										
Covariance parameter			Est	SE	Est	SE	Est	SE	Est	SE
Chamber			1.74	0.58	n/a	n/a	n/a	n/a	0.04	0.03
Block			n/a	n/a	n/a	n/a	n/a	n/a	-0.01	0.01
Chamber x Block			n/a	n/a	0.28	0.78	0.01	0.01	n/a	n/a

Note: bold indicates significant differences (α=0.05).

Table 4.2. ANOVA results for the fixed effects of population origin, light treatment, and their interactions, and the random effects of experimental chamber, block, and their interactions on *M. vimineum* days to anthesis, total biomass, aerial biomass, and root biomass. 'Est' is the covariance parameter estimate and 'SE' in the standard error of the covariance parameter estimate. 'n/a' specifies that Wald Z values could not be calculated due to negative covariance estimates, which indicates that the random effect was not significant.

Chapter 5

Research Implications and Future Directions

Abstract

In this chapter, I present some of the management implications of the research presented in the preceding chapters of this thesis. Specifically, I discuss how the information generated in these studies can be used to improve our evaluation of management strategies for *Microstegium vimineum*, especially biocontrol agents. I also discuss the importance of incorporating evolutionary processes into invasive plant prediction schemes. I present several avenues of new research that would build on the data presented in this thesis to extend our studies to include both demographic aspects of *M. vimineum* biology and refinement of our understanding of the evolutionary forces that contribute to invasion. Finally, I conclude with a more general discussion of how we might incorporate evolutionary thinking into invasion biology, and I offer some brief thoughts on how the new concept of 'novel ecosystems' may influence our approach to invasion biology.

Implications for Management of *M. vimineum* invasions

Although the experiments described in this thesis did not specifically evaluate potential management strategies for *M. vimineum*, I have nonetheless uncovered important details of the species' biology that have implications for its management. It has been noted that the design and success of control strategies, especially potential biological control agents, depends on knowing the origin, character, and geographical extent of genetic diversity within and among invasive populations (Valiant et al. 2007). Based on literature review, there are two known fungal pathogens of *M. vimineum*, either of which could potentially be considered for biocontrol. The first is the hyphomycete fungus *Pleurovularia polliniae*, which has been discovered to be a parasite on *M. vimineum* in Taiwan (Kirschner et al. 2002). The second is a newly emerging fungal pathogen *Bioparis* sp., discovered on *M. vimineum* in its invasive range (Kleczewski and Flory 2010). Of course, any potential biocontrol agent would need to be evaluated for potential damaging effects on non-target organisms.

The information presented in this thesis provides two important biological details of *M. vimineum* which could be useful in evaluating the potential of these, or any other, biocontrol agents. First, the population genetic structure measured in Chapter 3 showed that *M. vimineum* can be classified into three sub-regional groups. When testing biocontrol agents, it will be important to include *M. vimineum* samples from all three of these sub-regions, in case genetic elements that might affect the efficacy of biocontrol have been distributed in a pattern similar to that of the microsatellite loci assayed here.

Second, in Chapter 4, I presented evidence that *M. vimineum* has undergone phenological evolution driven by selective pressures to maximize reproduction at various latitudes. This evolution would only have been possible with underlying genetic variation that could be selected upon. It is also possible that *M. vimineum* individuals in the invasive range possess underlying genetic variation that may result in differential response to biocontrol agents. Analogously, *M. vimineum* might be able to evolve relatively quickly around any biocontrol agents deployed. The potential to evolve around a biocontrol agent does not necessarily preclude the benefit of that agent. However, a more complete understanding of evolutionary potential, as it relates to biocontrol may support certain specific biocontrol strategies. For example, if it were to be determined, after applying a biocontrol agent to multiple *M. vimineum* individuals from several regions, that there were resistance genes present in some regions but not others, then that biocontrol agent should only be used where the plant is susceptible. In any case, we will most likely have to institute a continuous program of biocontrol development and monitoring to successfully deploy such strategies. Furthermore, management priorities may need to be created to keep resistance genes out of susceptible areas, in order to preserve the biocontrol agent's efficacy in the susceptible sections of the plant's invasive range.

More generally, the reality that evolutionary processes are occurring during *M. vimineum* invasion should impact overall thinking about the factors affecting its range expansion. I showed that evolution of phenology is most likely necessary for the species' range expansion, especially northward.

There may be other traits that would allow the species to evolve novel genotypes, conferring fitness advantages during range expansion. Therefore it may be warranted to monitor for and avoid introduction of novel genotypes from the native range into the invasive range that might confer such adaptational advantages. For example, *M. vimineum* may reach a northern maximum, beyond which it cannot currently evolve the capacity to survive, because its standing genetic variation, or even mutational potential, will not present an evolutionary solution that allows for completion of its lifecycle below certain temperatures or under more northerly light regimes. If, as is supposed, the *M. vimineum* genetic stock currently present in North America derives from central China, the species may gain the ability to colonize further north than its current maximum, with the introduction of genetic material from further north in the species' native range. This could be true for a range of traits beyond those discussed in this thesis, as range expansion is likely to be facilitated by genetic elements for many physiological (e.g., heat/cold tolerances) and life history traits.

Predicting invasion

One of the long-standing goals of invasion biology has been to develop a predictive framework for determining which introduced species are likely to become successful invaders (Kolar and Lodge 2001). While there has been a debate as to whether or not a predictive framework for invasive species is possible at all (e.g., Williamson 1999), work to develop predictive frameworks

has continued. Certainly, progress in the area has been made (reviewed by Kolar and Lodge 2001). Currently, national governments (e.g., Australia) have developed and implemented schemes to predict plant weediness and invasiveness, via screening of plant introductions (Pheloung 1995). In fact, an economic analysis of application of the Australian scheme indicated that the system does produce net economic benefits, at least with regard to risk/benefit analysis of the program, as applied to the ornamental plant industry (Keller et al. 2006). However, both the Australian scheme and other proposed systems view invasiveness in terms of static traits that are used as predictive characters (e.g., clonal growth, high fecundity). One major caveat attached to that view is that it does not consider invasive traits as subject to adaptive evolutionary processes. Whitney and Gabler (2008) have surveyed the literature and various invasion predictive schemes to determine the depth of the problem. They found that of 29 predictive schemes proposed, 22 (76%) envision invasion traits as static entities. Of the seven schemes that are not fully static, only three recognize general adaptive potential, while the remainder account specifically for hybridization potential only. None of the schemes allows for adaptive evolution within the recipient communities, despite the fact that there are records in the peer-reviewed literature of at least 38 invasive species in which traits associated with invasive potential have undergone evolutionary change since invasion.

Based on the studies conducted in this thesis (see Chapter 4), *M. vimineum* can now be added to the list of invasive species that have undergone adaptive evolution in their introduced range, probably underlying the range

expansion. Evolutionary processes *per se* are important in determining invasive potential of many species and, echoing Whitney and Gabler (2008), we need better metrics of evolutionary potential that should, in turn, be incorporated into schemes predicting invasiveness. However, even if well quantified, how evolutionary potential translates into invasive success is likely to be highly uncertain. Especially where evolution is a heavy determinant of invasive success, predictive accuracy will never be 100%. In view of the fact that *M. vimineum* is not clonally propagated, has a weak root system, relatively few dispersal vectors, and is not known to be toxic or allelopathic (the usual features associated with invasive success), the point becomes even more salient. The relevant question then becomes: Is *M. vimineum* a typical example of an invasive plant? I would argue that by virtue of not fitting the usual paradigm of invasive characteristics (which is currently thought of in terms of static traits), *M. vimineum* does represent an atypical example. Certainly, as Whitney and Gabler (2008) tabulated, there are many other similar examples

There is much work still to be done in improving our prediction schemes. If the inclusion of evolutionary thinking can be widely incorporated into invasion predictive schemes, species like *M. vimineum* may become typical and predictable in a new paradigm. If evolutionary processes prove so unpredictable that predictive schemes cannot distinguish between invasive and non-invasive organisms, then predictive schemes that meet the societal needs, including economic concerns, may prove elusive. Although these questions remain open, there is currently reason to be optimistic that predictive schemes may be

beneficial, even if imperfect. After all, the early cost benefit analysis of the Australian scheme showed net benefit (Keller et al. 2006), even though that scheme does not account for evolutionary potential. It is possible that continued study of invasion biology will lead to more advanced schemes with an even better cost/benefit ratio when evolutionary processes are sufficiently understood to allow their inclusion in decision rubrics.

Lessons for Transgene Containment?

Cleistogamy has been proposed as a potential method of transgene containment, especially in crops within the Poaceae (Daniell 2002). Although no one has proposed creating a transgenic *M. vimineum*, the study of cleistogamous grasses, such as *M. vimineum*, may provide important biological details which can inform the discussion of potential transgene containment via cleistogamy. Cleistogamy may provide an effective barrier to gene flow via pollen, but it would still be possible for cleistogamous transgenic crop genes to escape by seed dispersal or occasional out-crossing. Escapee seed derived via cleistogamy would most likely initially retain their cleistogamous barrier to intercrossing with wild relatives, but novel genotypes (potentially with lower fidelity of cleistogamy) could still be generated by self-pollination of heterozygotes within the cleistogamous flower spike or the rare out-crossing event. Chapter 3 demonstrates that even with high rates of cleistogamy, which in most cases lead to allelic fixation within populations, even relatively small amounts of

chasmogamy may lead to genetic change and divergence over time. Furthermore, mutational innovation could arise, and be passed on to offspring, even during cleistogamous reproduction. The presence of haplotypic novelty and private alleles found only in the invasive range of *M. vimineum* in Chapter 3 could indicate persistent genotypes having arisen *de novo* via mutation. The demographic consequences of even a small degree of chasmogamy and/or mutational innovation are likely to be further enhanced if thusly derived novel genotypes are of superior fitness or increased chasmogamous flower production. Therefore, cleistogamy will only be a successful method of transgene containment if it is virtually 100% chasmogamy-free and/or if chasmogamously and mutationally derived novel genotypes are both infrequent and of inferior fitness.

Future research

Two of the main conclusions of this research are: (1) the mating system of *M. vimineum* gives rise to substantial genetic structure, with some genetic variation within populations (see Fig. 3.4), almost no heterozygosity, due to high rates of inbreeding, and large divergence among regional groupings; and (2) in its invasive range, there exists a cline of phenological variation along a latitudinal gradient that most likely indicates adaptive evolution. These results suggest several areas of future research.

First, although *M. vimineum* appears to exhibit a specific population genetic structure, as a result of its mixed cleistogamous/chasmogamous mating system, it is unclear whether that mating system *per se* confers an advantage, relative to cohabiting native competitors or other potential invaders. Determining if this specific mating scheme provides a generalizable advantage to invading organisms could be an important variable for inclusion in invasion prediction schemes. Therefore, experimental designs which can quantify the relative advantage this mating system may confer should be considered for a range of habitats. The genus *Microstegium* contains at least 20 additional species, of which 13 are present in China (Chen and Phillips 2008), the supposed source of *M. vimineum* invasions. It would be useful to survey the other species of *Microstegium*, which are morphologically quite similar, to determine the extent to which they possess different mating systems. It would be particularly interesting to find a congener that also has a mixed cleistogamous/chasmogamous mating system, but with a significantly different ratio of cleistogamy to chasmogamy than *M. vimineum*, especially if it is determined that congeners of *M. vimineum* have been introduced into North America along with *M. vimineum* yet have failed to establish or become invasive.

For example, *M. vimineum* plants generally have more cleistogamous flowers than chasmogamous flowers. If a congener with the opposite ratio could be found, then one could design direct competition experiments under a range of conditions to determine which mating system is advantageous in various habitats. Ideally, these kinds of experiments would have to be run over several

reproductive cycles and across a gradient of environments with differing rates of disturbance, since one would hypothesize that greater cleistogamous flower production may be less beneficial than greater chasmogamous flower production under changing environments, due to a decreased ability to generate novel genotypic combinations. Determining the conditions under which *M. vimineum*'s mating system is advantageous would be a first step towards demonstrating that the patterns of genetic structure determined in this thesis reflect performance characteristics related to invasion success as opposed to simply being the demographic pattern consequences of the mating system as assayed through a neutral marker system.

Second, although there were some signals in the population genetic dataset suggesting that *M. vimineum* in the United States may have its origin in eastern China, there was no robust evidence for the origin of invasion. This may be because sexual reproduction, followed by random drift or other stochastic processes, has altered genotypes sufficiently enough to make genetic similarity and assignment tests essentially meaningless, or it could reflect the fact that my sampling did not include the actual geographic range of the propagules giving rise to the North American invasion. In order to more definitively determine the origin of invasion, additional sampling throughout the native range of the species, but especially in the exact regions of central China which produced the porcelain that *M. vimineum* anecdotally followed to North America, should be undertaken. Furthermore, to account for the potential confounding effects of allelic recombination and segregation, it would be advisable to augment nuclear marker

assays with a uni-parentally inherited marker system such as chloroplast sequencing (e.g., Heinze 2007) or chloroplast microsatellites. Such an approach would greatly improve the chances of identifying the actual origin(s) of invasive populations. This information would be particularly valuable to evolutionary ecologists interested in cataloging the differing evolutionary trajectories of plants in their invasive range vs. their native ranges (e.g., Blossey and Notzold 1995).

Third, while the robust evidence of a phenological cline in *M. vimineum* is strong evidence for adaptive evolution in this species, it is not proof. It would be possible to prove the adaptive advantage of this evolutionarily derived pattern by conducting reciprocal common garden experiments in both the northern and southern regions of the invasive range using propagules collected in those respective regions. One could then measure seed production (fecundity) as a proxy for fitness. True fitness measurement would require tagging plants and their progeny (possibly using molecular markers) over multiple generations, to measure if more progeny (both in terms of abundance and biomass, or even proportion of inherited genetic material) of northern sourced plants survived in the northern environment, and likewise for the south.

Fourth, since phenological variation appears to be selected upon to confer a fitness advantage, it would be interesting to calculate the narrow sense heritability for the trait. This could be accomplished in several ways. For example, it would be fascinating to conduct controlled crosses of various permutations of parent plants from the populations sampled in Chapter 4 of this thesis. After

growing parents and progeny to first flowering, one could plot the mid-parent means for days to anthesis vs. the mid-offspring means. The slope of the best fit regression line would be the narrow sense heritability. Estimating the heritability of this trait would provide an initial point of evidence to understand how quickly the species can evolve these phenotypes, a potentially important element in understanding the duration of the species' lag phase as well as evolutionary potential for further range expansion.

Fifth, In light of climate change, *M. vimineum*, and other plant species, may be able to expand their ranges further toward the poles. However, this range expansion will not solely be a function of abotic tolerance regimes (frost dates, etc.) shifting northward, but will also be a function of these plant species' ability to adapt to new local light regimes (i.e., evolve phenologically). Depending on rates of climate change, plant species may or may not be able to evolve fast enough to keep up with climate conditions as they advance toward the poles (assuming they can disperse fast enough to keep up with climate change). *Microstegium vimineum* could be an excellent study species for quantifying potential rates of phenological evolution under various climate change regimes. This could be accomplished in a directed evolution experiment in controlled growth chambers. One could place several mature *M. vimineum* plants, representing several phenological phenotypes, in several growth chambers. Growth chambers could be set to mimic light regimes at several latitudes toward the northern range limit of *M. vimineum* and could even include conditions much farther north than its current range. In addition, each light treatment could be replicated several times.

The light treatment replications could each simulate a different date of first frost. In essence, there would be a nested factorial design of varying frost dates within latitudinal light treatments. Each chamber would have a fan to facilitate pollen flow among the chasmogamous flowers, so that even with minimal chasmogamy, some seeds should be heterozygotes representing novel genotypes. After the simulated frost date, seeds should be collected and tested for viability (a potential proxy metric for selection pressure). A random sampling of viable seeds should be grown out to determine the variation and average time to flowering resulting from each treatment (which should respond to different degrees of selection pressure in each light environment). These data could be used to calculate heritability. The seeds not removed for phenotype measurement could be replanted in the growth chambers again for another round of pollination and selection pressure. If this were to be repeated over three or more generations, and the selection pressures (in terms of days to simulated first frost) is increased for each generation, the maximum rate of phenological evolution (for the amount of genetic diversity initially included in the experiment) could be calculated based on the change of the average flowering time under each treatment. Such an experiment could provide valuable data about what kind of limitations to range expansion under climate change could be expected under various climate change rates. It would also be interesting to note if the species would be capable of evolving genotypes which could successfully reproduce under conditions typical of higher latitudes than the current range extents. Those data could help to predict the eventual northern limit of range expansion for the species.

Concluding Thoughts

In conducting the experiments presented in this thesis and reviewing the relevant literature, it became apparent that one of the most important tasks facing invasion biologists is incorporating evolutionary thinking into invasion studies. On a fundamental level, invasions are not at all different from the historical micro-evolutionary occurrences which led to range expansion before anthropogenic effects greatly increased the rate of species introduction into novel ranges. Therefore, the incorporation of evolutionary thinking into invasion biology will continue to provide a myriad of examples for theoretical evolutionary biologists to explore micro-evolutionary processes in a range of organisms, while providing useful information as invasion biologists continue to develop their field into a predictive science. However, as anthropogenic influence continues to alter the biosphere, the utility of the term 'invasive organisms', be they plant or otherwise, is likely to become diluted, as more and more ecosystems diverge from their pre-anthropogenic trajectories. It is increasingly likely that humankind will inhabit a world of 'novel ecosystems' (i.e., those containing new species assemblages arising from human action, environmental change, and the introduction of novel species; Hobbs et al. 2006). Depending on how we value the ecosystem services provided by these increasingly common 'novel ecosystems', we may choose to move beyond the pejorative associations inherent in the term 'invasion biology'. Nevertheless, the knowledge gained from empirical and theoretical studies of the various processes, incidences and systems of invasion will provide the

foundation for evolutionary studies in a world dominated by the ‘novel ecosystems’ we continue to create.

Appendix A - Raw allelic data and STRUCTURE analyses from the population genetic study

Table A.1. List of 108 haplotypes determined by microsatellite assay of 570 *M. vimineum* samples, not including multi-locus heterozygotes. Region A1 includes samples from Yunnan, China. Region A2 includes samples from the Shanghai and Zhe Jiang Provinces in China. Region A3 is Japan. Region US4 includes NY, NJ, and CT. Region US5 includes MD, PA, and Rockingham VA. Region US6 includes AL, AR, GA, Indiana, TN, MS, NC, SC, and Mecklenburg VA. West Virginian haplotypes were present in all three US regions. Ohio haplotypes were present in US5 and US6. Yellow shading indicates loci which were heterozygous in their diploid genotypes. "-9" indicates missing data.

Haplotype	Region	Locus										Copies in Dataset	From Genotype w/ 1 Heterzygous Locus	Populations w/ Haplotype
		MV01	MV03	MV10	MV09	MV05A	MV05B	MV06	MV07	MV08	MV02			
H001	A1	230	309	271	106	382	418	309	378	305	224	2	Yes	CN3
H002	A1	230	318	271	106	382	415	312	372	318	224	4	No	CN7
H003	A1	230	318	271	106	393	412	313	372	315	224	14	No	CN7
H004	A1	232	318	271	106	382	418	312	372	305	224	12	No	CN7
H005	A1	232	318	271	106	393	418	313	378	315	224	18	No	CN3
H006	A1	230	318	271	106	382	418	309	378	305	224	2	No	CN3
H007	A1	230	318	271	106	393	415	312	367	315	224	2	No	CN7
H008	A1	232	-9	-9	106	-9	-9	-9	-9	-9	224	2	No	CN3
H009	A1	232	-9	271	106	-9	-9	313	-9	-9	224	2	No	CN3
H010	A1	232	312	271	106	382	418	312	372	305	224	1	Yes	CN7
H011	A1	232	318	271	106	-9	-9	313	-9	-9	224	2	No	CN3
H012	A1	232	318	271	106	382	418	309	378	305	224	2	No	CN3
H013	A1	232	318	271	106	382	418	309	378	315	224	2	No	CN3
H014	A1	232	318	271	106	382	418	312	-9	-9	-9	2	No	CN7
H015	A1	232	318	271	106	382	418	313	-9	-9	-9	2	No	CN7
H016	A1	232	318	271	106	392	-9	313	378	315	224	2	No	CN3
H017	A1	232	318	271	106	393	418	-9	378	315	224	2	No	CN3
H018	A1	232	318	271	106	393	418	313	-9	315	224	2	No	CN3
H019	A1	232	321	271	106	382	418	312	372	305	224	1	Yes	CN7
H020	A2	227	341	283	123	397	428	315	-9	-9	224	8	No	CN11

Table A.1. (cont'd).

Haplotype	Region	Locus										Copies in Dataset	From Genotype w/ 1 Heterzygous Locus	Populations w/ Haplotype
		MV01	MV03	MV10	MV09	MV05A	MV05B	MV06	MV07	MV08	MV02			
H021	A2	227	341	283	123	397	428	315	372	315	224	12	No	CN12
H022	A2	227	341	283	123	397	428	315	372	316	224	8	No	CN11
H023	A2	230	318	271	106	382	418	309	378	305	224	2	Yes	CN4
H024	A2	238	318	272	121	389	415	309	372	321	224	12	No	CN15
H025	A2	240	312	271	117	394	428	345	372	315	226	10	No	CN17
H026	A2	244	316	271	133	394	415	303	372	326	226	20	No	CN13
H027	A2	246	312	271	125	394	415	347	372	326	224	6	No	CN16
H028	A2	260	309	271	133	380	424	333	372	324	224	16	No	CN14
H029	A2	-9	312	271	-9	394	415	-9	372	-9	224	2	No	CN16
H030	A2	-9	316	271	-9	-9	415	-9	372	326	226	2	No	CN13
H031	A2	227	341	283	123	397	428	315	375	315	224	2	No	CN12
H032	A2	227	316	271	133	-9	415	303	372	326	226	2	No	CN13
H033	A2	232	318	271	106	382	418	312	372	305	224	1	Yes	CN8
H034	A2	232	321	271	106	382	418	317	372	305	224	1	Yes	CN8
H035	A2	238	318	-9	121	389	415	309	372	321	-9	2	No	CN15
H036	A2	244	316	271	121	397	415	315	367	307	226	1	Yes	CN11
H037	A2	244	316	271	121	397	415	324	367	307	226	2	No	CN11
H038	A2	244	316	271	133	394	-9	303	372	326	226	2	No	CN13
H039	A2	246	312	271	125	394	415	345	372	326	224	1	Yes	CN16
H040	A3	235	309	271	121	386	424	345	382	315	224	36	No	J
H041	A3	235	309	271	125	394	418	315	370	324	224	6	No	J
H042	A3	235	370	262	121	394	428	315	375	318	224	2	No	J
H043	A3	244	316	271	121	397	415	324	367	307	226	1	Yes	CN12
H044	A3	246	312	271	125	394	415	347	372	326	224	1	Yes	CN17
H045	A3	246	312	271	125	394	415	347	-9	-9	224	2	No	CN16
H046	A3	250	316	271	117	397	415	345	372	305	226	2	No	CN16
H047	US4	235	344	271	137	383	418	313	375	316	224	14	No	NJ
H048	US4	235	358	271	129	394	424	315	-9	-9	226	14	No	NY, NJ

Table A.1. (cont'd).

Haplotype	Region	Locus										Copies in Dataset	From Genotype w/ 1 Heterzygous Locus	Populations w/ Haplotype
		MV01	MV03	MV10	MV09	MV05A	MV05B	MV06	MV07	MV08	MV02			
H049	US4	235	358	271	129	394	424	315	378	326	226	46	No	NY, NJ
H050	US4	-9	-9	271	129	-9	-9	-9	378	324	226	2	No	CT
H051	US4	-9	358	271	-9	394	424	-9	-9	-9	226	2	No	NY
H052	US4	235	344	271	137	383	418	313	-9	-9	224	2	No	NJ
H053	US4	235	358	-9	129	394	424	315	378	324	226	2	No	CT
H054	US4	235	358	271	129	394	424	315	378	324	226	34	No	WV, CT
H055	US5	229	312	271	129	386	428	336	370	-9	228	8	No	VA2
H056	US5	229	312	271	137	386	422	303	370	315	224	30	No	VA2, PA
H057	US5	229	334	271	137	394	428	312	375	324	226	4	No	MD
H058	US5	229	361	271	137	386	424	312	370	326	236	10	No	VA2
H059	US5	235	344	271	137	383	418	313	375	315	224	6	No	MD
H060	US5	-9	-9	271	137	-9	-9	-9	370	315	224	2	No	PA
H061	US5	229	312	271	129	386	428	336	370	315	224	1	Yes	MD
H062	US5	229	312	271	129	386	428	336	370	315	228	1	Yes	MD
H063	US5	229	312	271	129	386	428	336	370	315	228	2	No	PA
H064	US5	229	312	271	137	386	422	303	-9	-9	-9	2	No	VA2
H065	US5	229	312	271	137	386	422	303	370	-9	224	2	No	VA2
H066	US5	229	312	271	137	386	424	312	-9	-9	-9	1	Yes	VA2
H067	US5	229	361	271	137	386	424	312	-9	326	236	2	No	VA2
H068	US5	229	361	271	137	386	424	312	370	-9	236	2	No	MD
H069	US5	229	361	271	137	386	424	312	370	324	236	2	No	VA2
H070	US5	229	312	271	129	386	428	336	370	315	228	68	No	WV, VA2, PA, NC, MD
H071	US6	235	344	271	137	383	418	999	-9	-9	-9	2	No	OH
H072	US6	-9	-9	271	121	-9	-9	-9	375	315	224	4	No	KN2, GA
H073	US6	231	358	271	121	394	424	339	372	324	228	14	No	NC
H074	US6	244	309	271	125	383	428	336	375	315	224	4	No	GA

Table A.1. (cont'd).

Haplotype	Region	Locus										Copies in Dataset	From Genotype w/ 1 Heterzygous Locus	Populations w/ Haplotype
		MV01	MV03	MV10	MV09	MV05A	MV05B	MV06	MV07	MV08	MV02			
H075	US6	246	309	271	-9	383	428	336	375	315	224	6	No	MS1, KN6, GA
H076	US6	246	309	271	121	383	428	330	375	315	224	6	No	MS2
H077	US6	246	309	271	121	383	428	336	-9	-9	-9	6	No	VA1, KN4, AR
H078	US6	246	309	271	121	383	428	336	-9	-9	224	4	No	SC1
H079	US6	246	309	271	121	383	428	336	375	315	224	458	No	VA1, SC2, SC1, NC, MS2, MS1, KN6, KN5, KN4, KN3, KN2, KN1, IN, GA, AR, AL
H080	US6	246	309	271	121	383	428	336	375	316	224	28	No	SC1
H081	US6	-9	-9	271	129	-9	-9	-9	372	315	226	2	No	MS2
H082	US6	-9	309	271	-9	383	428	-9	-9	-9	224	2	No	SC1
H083	US6	229	312	271	129	386	428	336	370	315	228	1	Yes	MD
H084	US6	229	312	271	129	386	428	336	375	315	228	1	Yes	MD
H085	US6	229	361	271	137	386	424	312	-9	-9	-9	1	Yes	VA3
H086	US6	235	316	271	129	400	428	315	372	315	226	2	No	MS2
H087	US6	244	306	271	121	-9	428	333	375	315	224	2	No	MS2
H088	US6	244	309	271	121	383	428	336	375	315	224	2	No	GA
H089	US6	246	309	-9	121	383	428	336	375	315	224	2	No	AR
H090	US6	246	309	271	121	-9	-9	312	-9	-9	-9	1	Yes	IN
H091	US6	246	309	271	121	383	428	-9	-9	315	224	2	No	IN
H092	US6	246	309	271	121	383	428	-9	375	315	224	2	No	NC

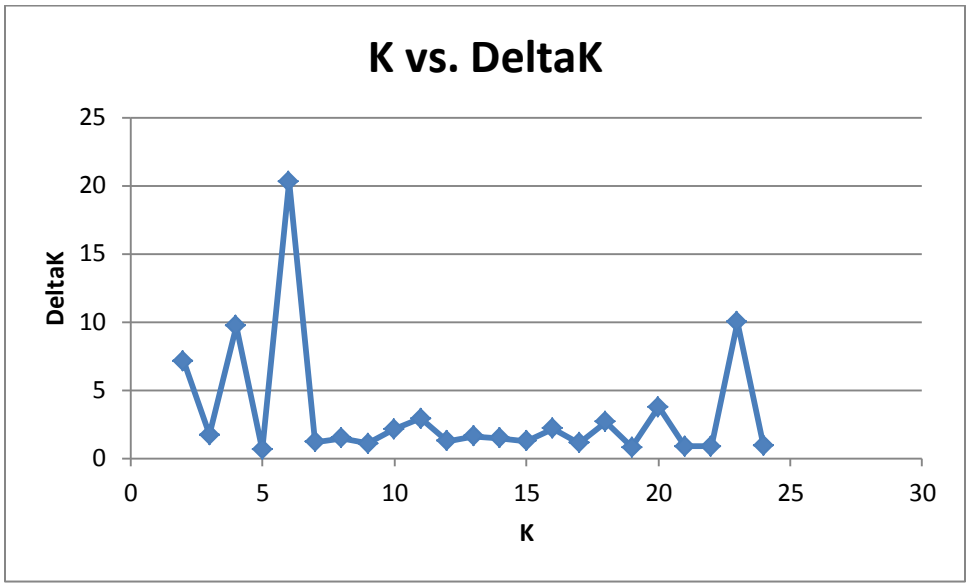
Table A.1. (cont'd).

Haplotype	Region	Locus										Copies in Dataset	From Genotype w/ 1 Heterzygous Locus	Populations w/ Haplotype
		MV01	MV03	MV10	MV09	MV05A	MV05B	MV06	MV07	MV08	MV02			
H093	US6	246	309	271	121	383	428	309	375	316	224	1	Yes	SC1
H094	US6	246	309	271	121	383	428	327	375	315	224	2	No	KN3
H095	US6	246	309	271	121	383	428	327	375	316	224	2	No	SC1
H096	US6	246	309	271	121	-9	-9	336	-9	-9	-9	1	No	IN
H097	US6	246	309	271	121	383	428	336	375	316	224	1	Yes	SC2
H098	US6	246	309	271	121	383	428	336	375	-9	-9	2	No	MS1
H099	US6	246	309	271	121	383	428	336	375	-9	224	2	No	SC1
H100	US6	246	309	271	121	383	428	336	375	316	-9	2	No	SC1
H101	US6	246	309	271	121	383	428	339	-9	-9	224	2	No	SC1
H102	US6	246	309	271	125	383	428	336	375	315	224	2	No	KN2
H103	US6	229	312	271	129	-9	-9	336	370	315	228	4	No	WV
H104	US6	229	312	271	121	386	428	336	370	315	228	1	Yes	WV
H105	US6	229	312	271	129	386	428	336	370	315	228	1	Yes	WV
H106	US6	229	312	271	129	386	428	338	370	315	228	1	Yes	WV
H107	US6	229	312	271	129	386	428	336	370	315	228	1	Yes	WV
H108	US6	235	344	271	137	383	418	338	375	315	224	50	No	WV, OH

Table A.2. Genotypes of the 8 *M. vimineum* samples, among the 570 total samples, which were heterozygous at more than 1 locus. Regions are as in Table A.1, but West Virginia has not been assigned a region. Yellow shading indicates heterozygous loci. “-9” indicates missing data.

Genotype	Region	Locus																				Populations w/ Genotype
		MV01		MV03		MV10		MV09		MV05A		MV05B		MV06		MV07		MV08		MV02		
G01	A2	227	248	312	341	271	283	123	137	394	397	415	428	315	327	-9	-9	-9	-9	228	228	CN11
G02	US5	229	229	309	361	271	271	121	137	386	386	424	424	312	312	370	370	324	324	236	236	VA2
G03	US5	229	229	334	361	271	271	137	137	386	394	424	428	312	312	370	375	324	324	226	236	MD
G04	WV	235	235	312	344	271	271	121	137	383	383	418	418	338	338	375	375	315	315	224	224	WV
G05	WV	235	246	309	309	271	271	121	121	383	383	428	428	315	336	375	375	315	315	224	224	WV
G06	A2	246	246	312	316	262	271	125	133	386	394	415	415	330	347	-9	-9	-9	-9	226	226	CN16
G07	A2	246	250	312	316	271	271	117	125	394	397	415	415	345	347	372	372	305	326	226	226	CN16
G08	A2	250	250	316	316	271	271	121	125	394	397	415	415	345	345	372	372	305	305	226	226	CN16

A.



B.

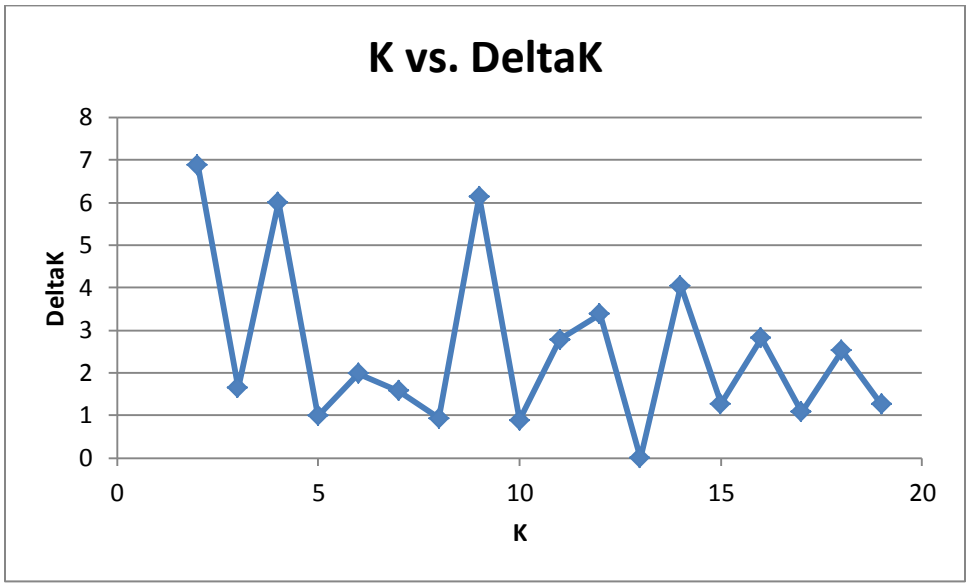
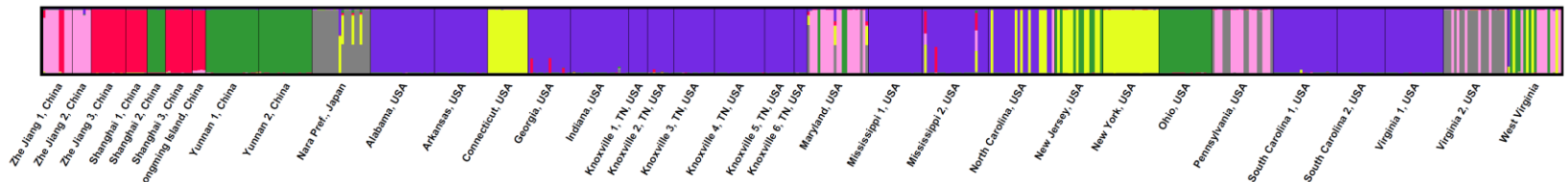
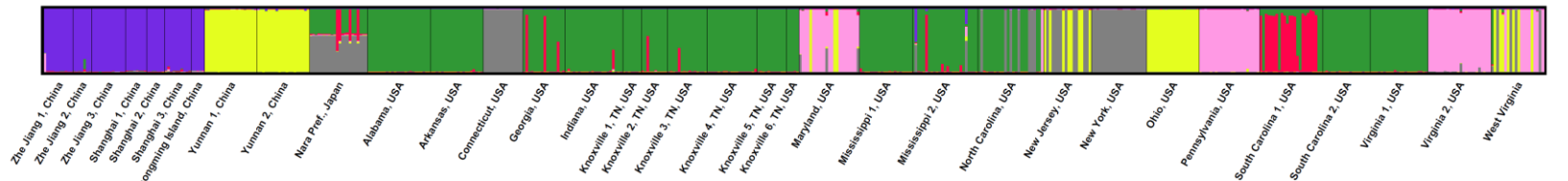


Figure A.1. Results of simulations for determining the appropriate K value to use in STRUCTURE simulations. A= 10,000 burnin and 10,000 MCMC reps after burnin; 20 runs each of K= 2-26. B= 50,000 burnin and 200,000 MCMC reps after burnin; 20 runs each for K=2-19

A.



B.



C.

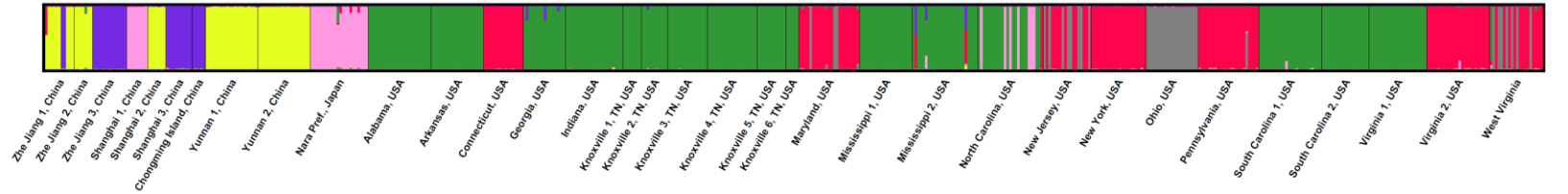
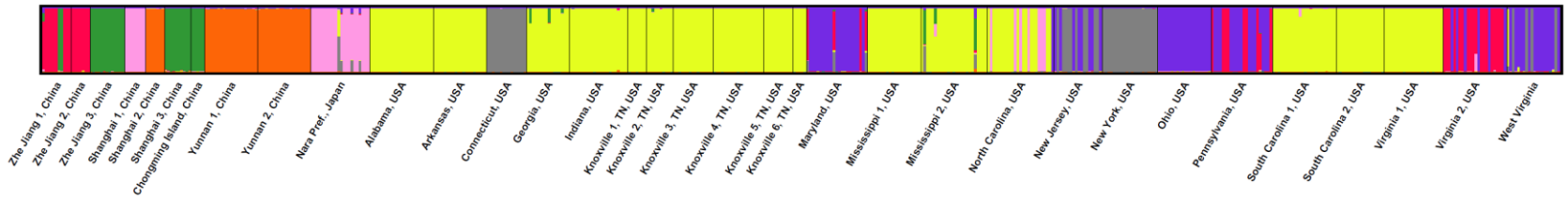
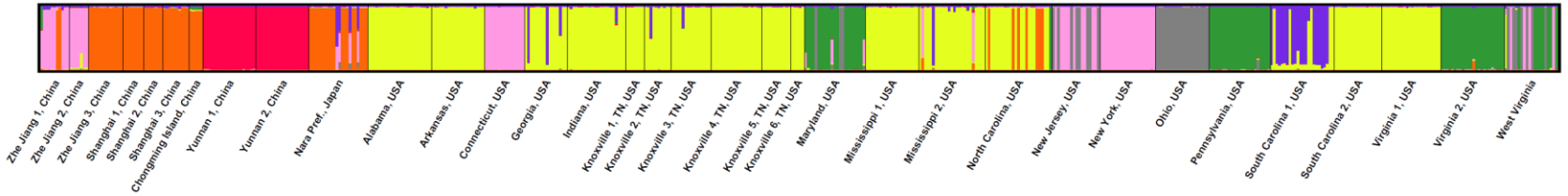


Figure A.2. Three representative STRUCTURE graphs for K=6 with 10000 burnin and 10000 MCMC reps of the 20 graphs generated.

A.



B.



C.

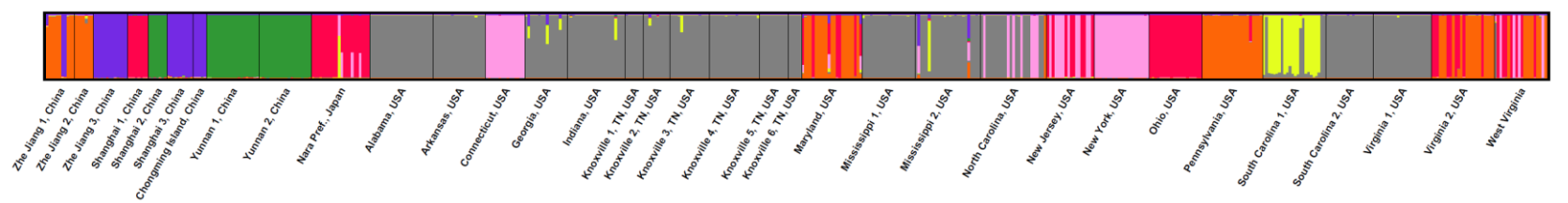
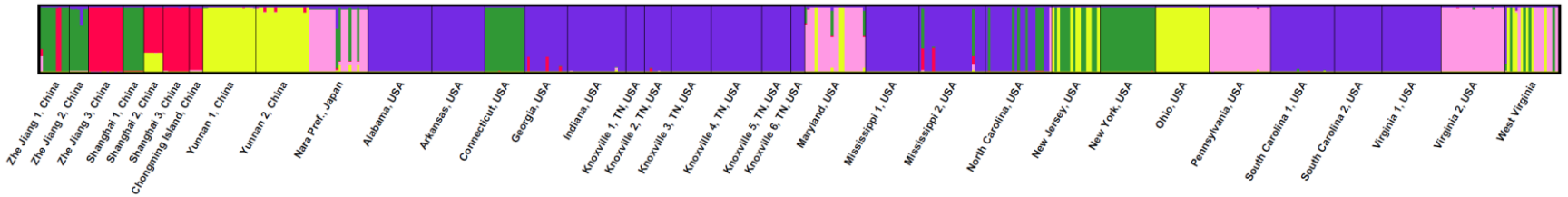
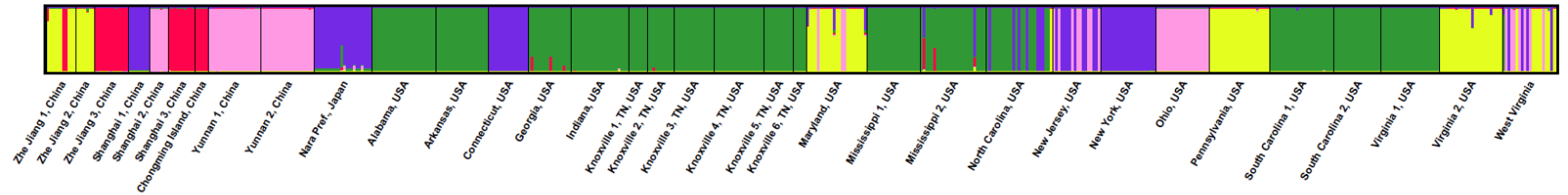


Figure A.3. Three representative STRUCURE graphs for K=7 with 10000 burnin and 10000 MCMC reps.

A.



B.



C.

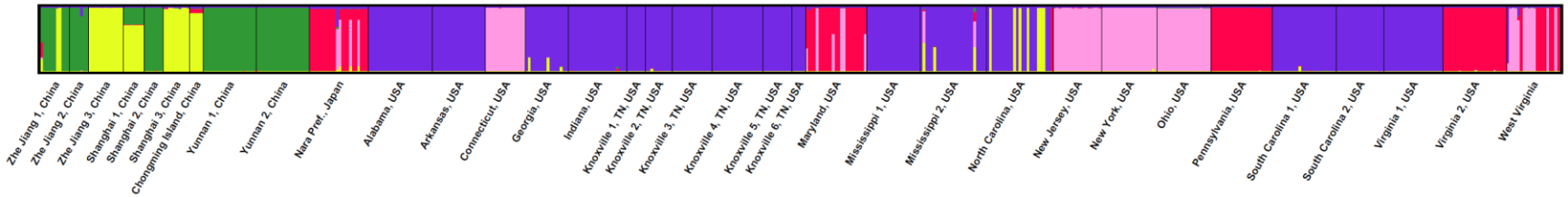
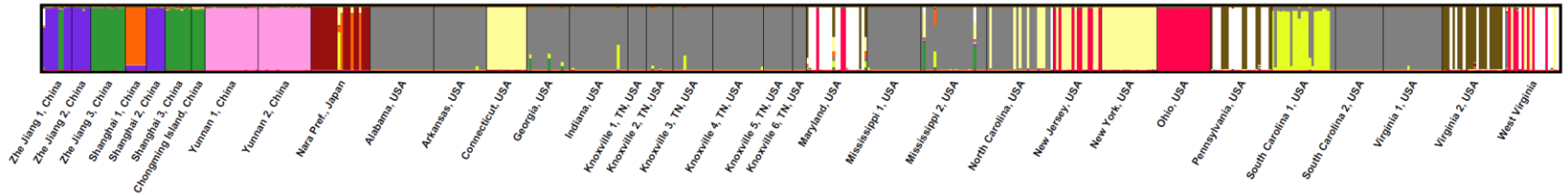
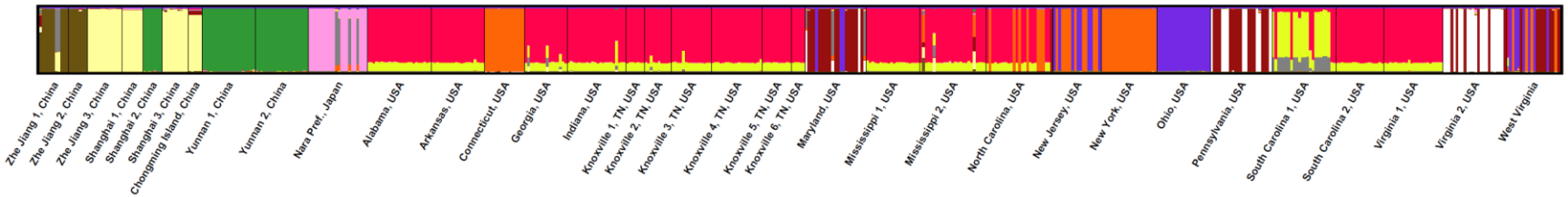


Figure A.4. Three representative STRUCURE graphs for K=5 with 50000 burnin and 200000 MCMC reps.

A.



B.



C.

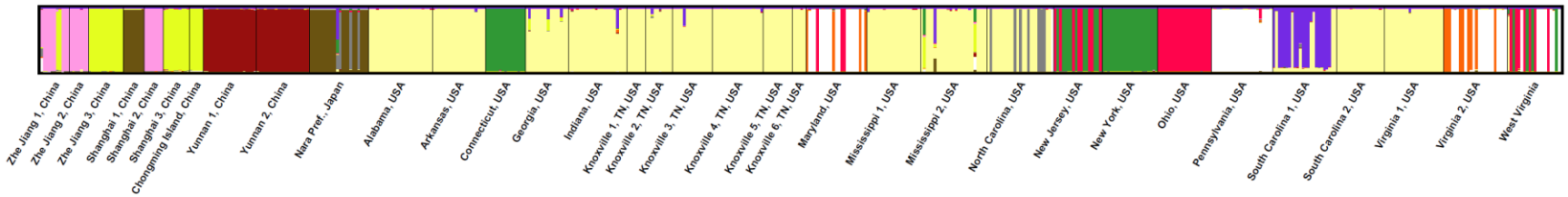
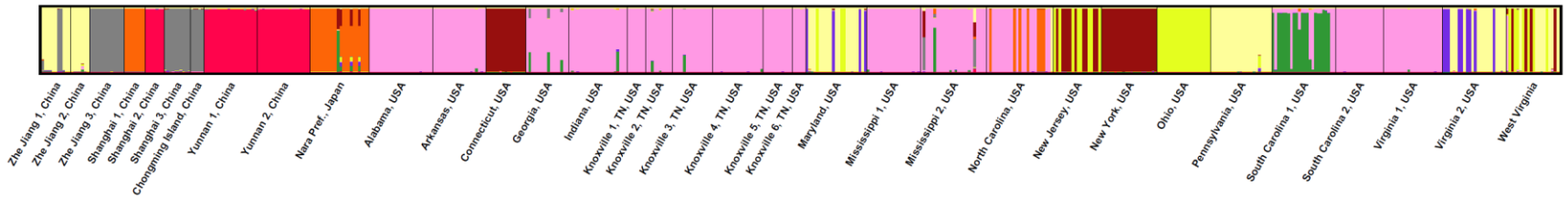
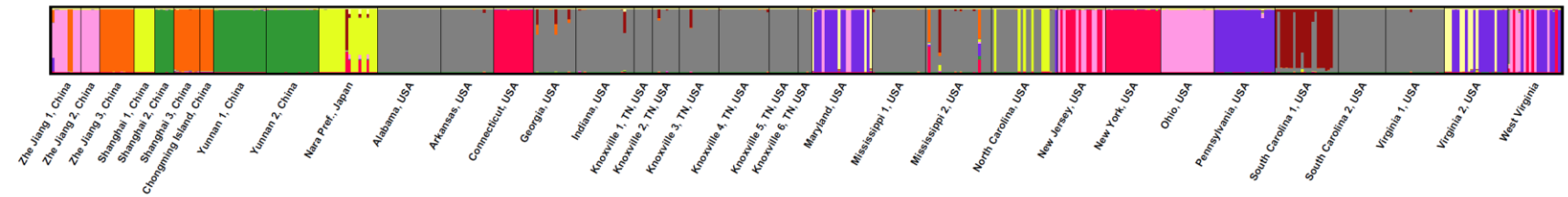


Figure A.5. Three representative STRUCURE graphs for K=11 with 50000 burnin and 200000 MCMC reps.

A.



B.



C.

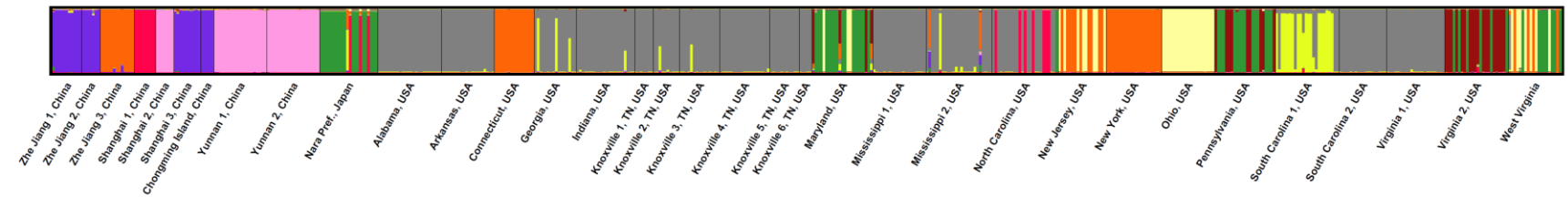


Figure A.6. Three representative STRUCURE graphs for K=9 with 50000 burnin and 200000 MCMC reps.

Appendix B

Raw data from the growth chamber experiment

Table B.1. Source locations of Asian seeds used in the growth chamber experiment but not reported in Chapter 4.

Pop Code	Country	Province/ Island	Prefecture	County	Nearest Town	Latitude	Longitude
CN1	China	Yunnan	Yuxi City	Xinping	Yaojie	23 55' 48" N	101 31' 24" E
CN2	China	Yunnan	Yuxi City	Xinping	Guasa	24 01' 56" N	101 34' 47" E
CN3	China	Yunnan	Yuxi City	Xinping	Mosha	23 45' 37" N	101 48' 45" E
CN4	China	Yunnan	Yuxi City	Xinping	Guishang	24 15' 48" N	101 23'46" E
CN5	China	Yunnan	Yuxi City	Yuan jiang	Lijiang	24 03' 24" N	101 57' 54" E
CN6	China	Yunnan	Yuxi City	Xinping	Shuitan	24 06' 39" N	101 51' 09" E
CN7	China	Yunnan	Yuxi City	Xinping	Zhelong	24 18' 10" N	101 21' 50" E
CN8	China	Yunnan	Yuxi City	Xinping	Pindiang	24 03' 32" N	101 57' 53" E
CN9	China	Yunnan	Yuxi City	Xinping	Xinhua	24 06' 39" N	101 51' 09" E
J1	Japan	Honshu	Nara		Akabane	34.51408 N	136.010417 E

Table B.2. Daylength settings used in growth chamber experiment. Lights always went on at 0700.

Week	Date	Chamber 1 (South 1)	Chamber 2 (South 2)	Chamber 3 (North 1)	Chamber 4 (North 2)
		Lights off (24h clock)	Lights off (24h clock)	Lights off (24h clock)	Lights off (24h clock)
1	9/28/2010	2059	2059	2205	2205
2	10/5/2010	2103	2103	2212	2212
3	10/12/2010	2106	2106	2217	2217
4	10/19/2010	2107	2107	2218	2218
5	10/26/2010	2105	2105	2215	2215
6	11/2/2010	2102	2102	2210	2210
7	11/9/2010	2057	2057	2201	2201
8	11/16/2010	2051	2051	2151	2151
9	11/23/2010	2043	2043	2138	2138
10	11/30/2010	2033	2033	2123	2123
11	12/7/2010	2023	2023	2106	2106
12	12/14/2010	2012	2012	2052	2052
13	12/21/2010	2000	2000	2034	2034
14	12/28/2010	1948	1948	2014	2014
15	1/4/2011	1936	1936	1956	1956
16	1/11/2011	1923	1923	1935	1935
17	1/18/2011	1910	1910	1913	1913
18	1/25/2011	1857	1857	1853	1853
19	2/1/2011	1842	1842	1834	1834
20	2/8/2011	1830	1830	1814	1814
21	2/15/2011	1818	1818	1754	1754
22	2/22/2011	1806	1806	1735	1735
23	3/1/2011	1755	1755	1717	1717
24	3/8/2011	1744	1744	1701	1701
25	3/15/2011	1733	1733	1645	1645
26	3/22/2011	1726	1726	1631	1631
27	3/29/2011	1719	1719	1619	1619
28	4/5/2011	1715	1715	1611	1611

Table B.3. Growth chamber block arrangement and plant randomization in each growth chamber. Blocks were numbered 1-5 starting on the upper left. The growth chamber door would have been on the left between blocks 1 and 5.

Growth Chamber 1

CN6-2	CN2-1	DE-1	VA1-1	CT-1	CN6-1	NC-1	VA1-2
CN7-1	CN1-1	J1-1	VA2-1	PA-2	CN8-2	J1-2	WV-2
PA-1	CN5-2	VA2-2	WV-1	CN9-2	CN1-2	CN8-1	CT-2
DE-2	CN3-2	CN5-1	CN4-2	NJ-1	MD-1	CN3-1	NJ-2
MD-2	NC-2	CN2-2	CN4-1	SC-2	CN7-2	SC-1	CN9-1

CN6-2	WV-2	CT-2	CN4-2	PA-2	CN8-1	CN2-2	NC-2
DE-2	VA2-2	MD-1	WV-1	PA-1	CN2-1	CN8-2	CN7-2
NJ-1	CN1-2	CN3-2	VA1-2	CN9-2	CN6-1	J1-1	SC-2
CN9-1	CN5-2	CN5-1	VA1-1	CN7-1	J1-2	SC-1	CN4-1
NC-1	DE-1	CN1-1	MD-2	NJ-2	CT-1	CN3-1	VA2-1

NJ-1	CN6-2	CN2-1	CN3-1	CN7-2	J1-2	NJ-2	CN9-1
PA-1	J1-1	VA2-1	CN8-1	SC-2	CN1-2	CN3-2	CN7-1
VA2-2	SC-1	CN5-2	CN2-2	CT-1	CN1-1	CN6-1	CN9-2
CN8-2	VA1-1	MD-1	NC-2	MD-2	DE-1	CT-2	CN4-2
DE-2	NC-1	WV-1	PA-2	CN4-1	VA1-2	WV-2	CN5-1

DE-2	PA-2	NJ-2	NC-2	CN4-1	CN8-1	J1-2	CN7-1
VA2-2	CN8-2	CN5-1	CN5-2	CN7-2	CN3-2	CT-1	SC-1
NJ-1	CN6-1	CN2-1	CN3-1	DE-1	PA-1	CN6-2	MD-1
WV-2	CN9-1	NC-1	CT-2	WV-1	VA2-1	VA1-2	CN2-2
VA1-1	SC-2	CN4-2	CN1-1	MD-2	CN9-2	J1-1	CN1-2

DE-2	SC-1	MD-2	NC-2	CT-2	NJ-2	CN8-1	CN7-2
CN6-1	NJ-1	CN3-2	CT-1	CN1-2	VA2-2	CN8-2	J1-1
WV-1	J1-2	DE-1	CN7-1	CN2-1	CN9-1	CN5-2	CN3-1
VA2-1	NC-1	CN5-1	WV-2	CN4-2	VA1-1	CN6-2	MD-1
CN9-2	PA-2	CN4-1	SC-2	CN1-1	CN2-2	VA1-2	PA-1

Growth Chamber 2

NC-1	CN6-2	DE-2	CN5-1	SC-2	J1-1	VA1-1	MD-2
CN4-1	CN9-1	CN7-2	J1-2	CN3-2	CN2-1	NC-2	CN8-2
CN5-2	SC-1	CN1-1	CT-2	PA-2	WV-1	VA1-2	CN8-1
CN9-2	CN1-2	PA-1	VA2-1	CN6-1	NJ-1	CN2-2	MD-1
NJ-2	CN3-1	WV-2	CN4-2	VA2-2	DE-1	CN7-1	CT-1

CN1-1	MD-2	SC-1	CN6-2	VA2-2	WV-1	CN2-2	MD-1
CN9-2	PA-2	VA1-1	CN7-1	NJ-2	CN3-2	CN8-2	CN8-1
CN4-1	CN2-1	VA2-1	CN5-2	NJ-1	CN1-2	SC-2	CN4-2
DE-2	WV-2	VA1-2	CT-1	NC-1	J1-2	PA-1	CT-2
CN6-1	CN3-1	CN7-2	CN5-1	CN9-1	NC-2	J1-1	DE-1

CN8-1	CN7-1	CN5-2	PA-1	J1-1	CN4-1	PA-2	CT-1
VA2-2	CN9-2	CN3-1	CT-2	CN7-2	CN4-2	CN1-1	DE-2
VA2-1	J1-2	CN9-1	DE-1	SC-1	SC-2	CN5-1	MD-1
CN6-1	VA1-1	NJ-2	NC-1	CN2-1	CN3-2	CN1-2	NC-2
VA1-2	WV-1	NJ-1	CN6-2	CN8-2	MD-2	CN2-2	WV-2

CN9-2	CN5-1	MD-1	VA2-2	CN1-2	DE-1	CN2-1	NC-2
VA1-1	CN6-2	CN4-2	NC-1	CN3-1	DE-2	CN6-1	SC-1
J1-1	NJ-1	CN1-1	CN2-2	CT-2	CN3-2	VA1-2	J1-2
VA2-1	CN9-1	WV-1	CN7-1	CN7-2	CN4-1	SC-2	CN5-2
NJ-2	MD-2	WV-2	CT-1	CN8-1	PA-1	CN8-2	PA-2

DE-1	CN7-2	CN4-2	CN3-2	CN4-1	SC-2	CN9-2	CN3-1
WV-2	CN1-1	CN6-1	PA-1	VA1-1	VA2-1	J1-2	CN8-2
CN1-2	CN7-1	CT-1	CT-2	NC-2	NJ-1	CN6-2	WV-1
VA2-2	J1-1	DE-2	NC-1	CN2-2	CN2-1	SC-1	NJ-2
VA1-2	CN5-2	PA-2	MD-1	CN5-1	CN9-1	MD-2	CN8-1

Table B.3. (cont'd)

Growth Chamber 3

CN7-1	WV-2	CN1-1	CN1-2	VA2-2	MD-1	CN7-2	CN3-2
PA-1	CT-2	NJ-1	CN4-1	NC-1	J1-1	CN4-2	NC-2
PA-2	CN8-2	CN5-1	WV-1	SC-1	CN5-2	CN6-2	CN2-2
CN2-1	DE-1	SC-2	CT-1	CN9-1	MD-2	NJ-2	VA2-1
DE-2	CN9-2	CN8-1	CN6-1	CN3-1	J1-2	VA1-1	VA1-1

CN8-2	J1-2	DE-2	CN5-2	CN7-2	PA-1	CN7-1	CN4-2
CN1-1	CN2-1	MD-2	SC-1	NC-1	NJ-2	SC-2	CN8-1
DE-1	PA-2	CN3-2	CT-1	J1-1	CN4-1	VA1-2	MD-1
CN9-2	WV-2	VA2-1	CN3-1	VA1-1	CN5-1	VA2-2	CT-2
CN1-2	CN2-2	NC-2	CN6-1	WV-1	CN9-2	NJ-1	CN6-2

SC-2	VA1-2	CN1-2	NC-2	PA-2	CT-2	VA2-2	DE-2
WV-1	NJ-2	CN2-2	CN9-2	CN3-1	CN8-2	PA-1	WV-2
DE-1	MD-2	CN6-2	J1-2	VA1-1	CN6-1	CN7-2	CN8-1
NC-1	CN1-1	CN4-1	CN2-1	MD-1	CN9-1	CN4-2	CT-1
CN3-2	NJ-1	SC-1	CN5-2	CN7-1	VA2-1	J1-1	CN5-1

SC-1	CN3-2	VA1-1	VA2-1	CN1-1	CN9-2	CT-2	VA1-2
J1-2	CN4-2	MD-2	CN8-1	CN1-2	NJ-2	CN2-1	CN7-2
CN7-1	DE-1	CN8-2	DE-2	NC-1	CN4-1	CN9-1	VA2-2
SC-2	NC-2	CN6-1	MD-1	CN5-2	CN5-1	WV-2	CN3-1
PA-2	NJ-1	CN2-2	J1-1	WV-1	PA-1	CN6-2	CT-1

PA-1	J1-2	CN1-2	CN8-2	NJ-1	CN1-1	WV-2	PA-2
CN3-2	CT-1	SC-2	CN7-2	CN6-2	NJ-2	CT-2	CN8-1
VA1-1	CN9-2	CN5-1	DE-1	VA2-2	CN4-1	CN4-2	VA1-2
CN2-1	MD-1	DE-2	J1-1	NC-2	CN2-2	CN5-2	CN7-1
WV-1	VA2-1	CN6-1	NC-1	CN9-1	SC-1	CN3-1	MD2

Growth Chamber 4

CN8-2	WV-1	VA1-2	CN1-2	CN2-1	MD-2	DE-1	CN1-1
WV-2	NJ-1	MD-1	SC-2	NC-1	CN9-1	CN8-1	CN3-2
CN7-1	PA-1	CN6-2	CT-2	NJ-2	CN6-1	VA1-1	CN2-2
CN4-2	CN9-2	VA2-2	CN5-1	NC-2	DE-2	J1-2	CN5-2
VA2-1	SC-1	CT-1	CN4-1	PA-2	CN7-2	J1-1	CN3-1

NC-2	CN5-1	CN2-1	PA-1	NC-1	DE-2	CN9-1	SC-2
CN2-2	J1-1	CN3-1	VA1-1	CN8-2	NJ-1	VA2-2	CN7-1
CN9-2	VA2-1	CT-2	CN4-2	CN1-2	CN5-2	CN3-2	WV-1
MD-2	DE-1	CN1-1	CN7-2	CN6-1	CN8-1	VA1-2	WV-2
PA-2	NJ-2	CT-1	CN4-1	MD-1	J1-2	CN6-2	SC-1

CN9-2	CN2-1	CN7-2	CN2-2	CN6-2	VA2-2	J1-1	MD-1
CN1-2	CN3-1	PA-2	CT-1	CN4-1	MD-2	NJ-1	CN8-1
CN7-1	CN4-2	CN6-1	VA2-1	J1-2	SC-1	NC-1	DE-1
CN3-2	WV-2	PA-1	CT-2	SC-2	NJ-2	CN5-2	CN5-1
CN1-1	VA1-2	VA1-1	DE-2	WV-1	CN9-1	NC-2	CN8-2

DE-1	CN9-1	VA2-1	VA1-2	PA-1	CN7-2	MD-1	CN5-1
J1-2	NJ-1	DE-2	WV-1	CN6-1	VA2-2	CN2-1	CN8-1
CN1-2	NJ-2	CN3-2	CN2-2	MD-2	NC-1	WV-2	SC-1
CT-1	VA1-1	CN1-1	PA-2	CN4-1	CN7-1	NC-2	CN8-2
J1-1	CN6-2	CN3-1	CN5-2	CN4-2	CN9-2	SC-2	CT-2

VA2-1	J1-2	CN6-2	CN2-2	NJ-2	VA2-2	CN6-1	CN4-1
VA1-2	CN7-1	MD-1	CT-2	CN5-2	J1-1	CT-1	CN9-1
NC-2	CN3-2	CN2-1	NJ-1	CN7-2	NC-1	CN9-2	CN3-1
CN8-2	CN5-1	SC-2	DE-1	PA-2	CN8-1	PA-1	VA1-1
CN1-2	SC-1	DE-2	WV-1	WV-2	CN4-2	MD-2	CN1-1

Table B.4. Raw data of U.S. sourced plants used in the growth chamber experiment sorted by population, rep, chamber, and block. Senescence, inflorescence day (i.e., day of first emergence of immature inflorescence), and flowering day (i.e., day of first anthesis) are measured in number of days or weeks since seeds were germinated. Biomasses, height and terminal inflorescence counts were measured at senescence. GPS coordinates for each population are presented in Chapter 4. Dates are in the winter of 2010-11. Chamber 1 and 2 are the southern light treatment. Chamber 3 and 4 are the northern light treatment.

Pop	Rep	Chamber	Block	Senescence (wk)	Root Biomass (g)	Aerial Biomass (g)	Total Biomass (g)	Final Height (cm)	Infl. Date	Flowering Date	Infl. Day	Flowering Day	# Term Infl.
CT	1	1	1	14	1.005	2.1	3.105	99	30-Nov	1-Dec	63	64	12
CT	1	1	2	14	0.929	2.8	3.729	105	7-Dec	9-Dec	70	72	11
CT	1	1	3	14	0.755	3.4	4.155	108	4-Dec	6-Dec	67	69	17
CT	1	1	4	14	0.552	2.5	3.052	107	8-Dec	10-Dec	71	73	9
CT	1	1	5	14	1.154	3	4.154	111	4-Dec	6-Dec	67	69	12
CT	1	2	1	14	0.601	2	2.601	84	4-Dec	7-Dec	67	70	7
CT	1	2	2	14	0.882	2	2.882	97	7-Dec	9-Dec	70	72	8
CT	1	2	3	14	1.004	2.6	3.604	86	7-Dec	9-Dec	70	72	11
CT	1	2	5	14	0.77	2.5	3.27	114	4-Dec	6-Dec	67	69	8
CT	1	3	1	15	0.904	3.7	4.604	100	15-Dec	16-Dec	78	79	17
CT	1	3	2	15	1.328	4.3	5.628	108	25-Dec	29-Dec	88	92	11
CT	1	3	3	15	0.969	2.8	3.769	106	22-Dec	23-Dec	85	86	13
CT	1	3	4	15	0.618	3.1	3.718	102	17-Dec	19-Dec	80	82	11
CT	1	3	5	15	0.961	3.1	4.061	96	15-Dec	16-Dec	78	79	13
CT	1	4	1	16	1.467	4	5.467	96	15-Dec	19-Dec	78	82	10
CT	1	4	2	15	1.63	4.4	6.03	113	21-Dec	23-Dec	84	86	12
CT	1	4	3	15	1.156	3.1	4.256	116	28-Dec	28-Dec	91	91	12
CT	1	4	4	16	1.052	5.6	6.652	110	24-Dec	26-Dec	87	89	19
CT	1	4	5	16	1.11	2.8	3.91	107.5	19-Dec	21-Dec	82	84	11

CT	2	1	1	14	1.079	3.7	4.779	106	3-Dec	5-Dec	66	68	22
CT	2	1	2	14	0.932	3.3	4.232	122	1-Dec	2-Dec	64	65	10
CT	2	1	3	14	1.261	5.1	6.361	116	7-Dec	8-Dec	70	71	14
CT	2	1	4	14	1.245	4	5.245	105	7-Dec	9-Dec	70	72	18
CT	2	1	5	14	0.547	1.9	2.447	108	2-Dec	3-Dec	65	66	6
CT	2	2	1	14	0.639	2.6	3.239	109	7-Dec	11-Dec	70	74	7
CT	2	2	2	14	0.894	3.1	3.994	101	7-Dec	9-Dec	70	72	8
CT	2	2	3	14	1.004	2.8	3.804	100	6-Dec	8-Dec	69	71	8
CT	2	2	4	14	0.857	2.4	3.257	92	7-Dec	11-Dec	70	74	7
CT	2	2	5	14	1.307	4.5	5.807	106	4-Dec	6-Dec	67	69	12
CT	2	3	1	15	0.816	2.3	3.116	89.5	13-Dec	13-Dec	76	76	11
CT	2	3	2	15	0.632	0.7	1.332	91.5	17-Dec	19-Dec	80	82	9
CT	2	3	3	15	1.331	5.2	6.531	113	17-Dec	21-Dec	80	84	16
CT	2	3	4	15	0.969	4.3	5.269	103	19-Dec	19-Dec	82	82	13
CT	2	3	5	15	0.696	3.2	3.896	105.5	13-Dec	14-Dec	76	77	14
CT	2	4	1	16	0.771	2.5	3.271	100	22-Dec	24-Dec	85	87	8
CT	2	4	2	15	1.39	3.9	5.29	103	21-Dec	22-Dec	84	85	12
CT	2	4	3	16	0.65	2.3	2.95	98	24-Dec	25-Dec	87	88	8
CT	2	4	4	16	1.1	4.4	5.5	75	24-Dec	26-Dec	87	89	15
CT	2	4	5	15	0.399	4	4.399	116	19-Dec	19-Dec	82	82	16
DE	1	1	1	15	0.729	3.4	4.129	113	2-Dec	12-Dec	65	75	21
DE	1	1	2	16	0.614	3.7	4.314	107	20-Dec	21-Dec	83	84	7
DE	1	1	3	15	0.933	5.1	6.033	97	17-Dec	19-Dec	80	82	23
DE	1	1	4	15	0.981	4.2	5.181	95	25-Dec	26-Dec	88	89	12
DE	1	1	5	14	1.041	3.2	4.241	107	10-Dec	13-Dec	73	76	24
DE	1	2	1	15	0.634	3.7	4.334	115	20-Dec	22-Dec	83	85	7
DE	1	2	2	15	0.991	3	3.991	85	11-Dec	14-Dec	74	77	28
DE	1	2	3	15	0.537	2	2.537	83	23-Dec	24-Dec	86	87	7

DE	1	2	4	15	0.919	3.8	4.719	100	11-Dec	13-Dec	74	76	22
DE	1	2	5	12	0.517	3.4	3.917	78	20-Dec	23-Dec	83	86	6
DE	1	3	1	16	0.652	3.2	3.852	73.5	3-Jan	8-Jan	97	102	11
DE	1	3	2	15	1.067	4	5.067	92	14-Dec	14-Dec	77	77	23
DE	1	3	3	15	0.426	1.6	2.026	77	17-Dec	21-Dec	80	84	24
DE	1	3	4	16	0.867	2.7	3.567	89	17-Dec	21-Dec	80	84	22
DE	1	3	5	19	0.415	3.6	4.015	102	30-Dec	2-Jan	93	96	6
DE	1	4	1	19	0.769	4.2	4.969	121	6-Jan	10-Jan	100	104	11
DE	1	4	3	20	0.725	3.7	4.425	101	12-Jan	15-Jan	106	109	14
DE	1	4	4	20	0.582	3.9	4.482	86	11-Jan	15-Jan	105	109	7
DE	1	4	5	15	0.823	3.3	4.123	75	19-Dec	19-Dec	82	82	36
DE	2	1	1	15	0.74	2.8	3.54	104	11-Dec	13-Dec	74	76	21
DE	2	1	2	15	0.972	5.7	6.672	106	27-Dec	27-Dec	90	90	14
DE	2	1	3	15	0.676	4.8	5.476	87	20-Dec	23-Dec	83	86	15
DE	2	1	4	16	0.886	4.1	4.986	115	17-Dec	23-Dec	80	86	13
DE	2	1	5	15	0.502	2.8	3.302	111	21-Dec	24-Dec	84	87	10
DE	2	2	1	15	0.583	2.9	3.483	106.5	21-Dec	23-Dec	84	86	33
DE	2	2	2	15	0.611	2.5	3.111	93.5	17-Dec	22-Dec	80	85	7
DE	2	2	3	15	0.701	2.7	3.401	102	23-Dec	25-Dec	86	88	10
DE	2	2	4	15	0.683	3.4	4.083	87.5	20-Dec	24-Dec	83	87	16
DE	2	2	5	15	0.433	1.8	2.233	91	20-Dec	25-Dec	83	88	10
DE	2	3	1	19	0.736	5	5.736	93	3-Jan	8-Jan	97	102	12
DE	2	3	2	16	0.875	3.9	4.775	108	28-Dec	29-Dec	91	92	12
DE	2	3	3	19	0.789	6.5	7.289	129	10-Jan	11-Jan	104	105	16
DE	2	3	4	16	0.635	2.2	2.835	81	21-Dec	22-Dec	84	85	23
DE	2	3	5	19	0.797	3.8	4.597	94	30-Dec	5-Jan	93	99	6
DE	2	4	1	16	1.158	3.2	4.358	91	19-Dec	21-Dec	82	84	19
DE	2	4	2	20	1.083	6.1	7.183	96	10-Jan	17-Jan	104	111	7

DE	2	4	3	16	1.164	2.7	3.864	67	27-Dec	28-Dec	90	91	18
DE	2	4	4	15	1.224	4.7	5.924	85	18-Dec	19-Dec	81	82	44
DE	2	4	5	19	0.633	4.6	5.233	101	6-Jan	12-Jan	100	106	10
MD	1	1	1	15	1.215	5.1	6.315	96	15-Dec	19-Dec	78	82	20
MD	1	1	2	15	1.082	4.4	5.482	110	17-Dec	20-Dec	80	83	16
MD	1	1	3	15	0.952	4.5	5.452	97	23-Dec	26-Dec	86	89	12
MD	1	1	4	15	1.285	5.9	7.185	102	19-Dec	23-Dec	82	86	21
MD	1	1	5	15	1.749	6.6	8.349	119	21-Dec	24-Dec	84	87	17
MD	1	2	1	15	0.899	3.3	4.199	107	17-Dec	23-Dec	80	86	10
MD	1	2	2	15	1.287	6.7	7.987	113.5	2-Dec	22-Dec	65	85	19
MD	1	2	3	15	0.803	3.5	4.303	96	19-Dec	22-Dec	82	85	18
MD	1	2	4	15	0.739	3.5	4.239	98	20-Dec	22-Dec	83	85	13
MD	1	2	5	16	0.905	3.9	4.805	99	20-Dec	25-Dec	83	88	17
MD	1	3	1	19	1.484	5.7	7.184	113	6-Jan	10-Jan	100	104	17
MD	1	3	2	19	1.224	4.5	5.724	118	5-Jan	7-Jan	99	101	12
MD	1	3	3	19	0.969	4.4	5.369	95	5-Jan	11-Jan	99	105	13
MD	1	3	4	18	0.935	5.2	6.135	104	6-Jan	10-Jan	100	104	10
MD	1	3	5	19	1.09	6	7.09	95	5-Jan	7-Jan	99	101	12
MD	1	4	1	18	1.096	5.5	6.596	107	3-Jan	6-Jan	97	100	11
MD	1	4	2	19	1.589	6.4	7.989	87	10-Jan	12-Jan	104	106	17
MD	1	4	3	19	2.362	8.9	11.262	144	9-Jan	12-Jan	103	106	24
MD	1	4	4	19	1.684	6	7.684	107	9-Jan	11-Jan	103	105	18
MD	1	4	5	19	1.234	5	6.234	107	10-Jan	12-Jan	104	106	19
MD	2	1	1	16	0.697	3.8	4.497	108.5	20-Dec	23-Dec	83	86	15
MD	2	1	2	15	1.29	5.1	6.39	106	23-Dec	26-Dec	86	89	14
MD	2	1	3	15	1.36	5.8	7.16	100	23-Dec	27-Dec	86	90	15
MD	2	1	4	16	1.559	6.1	7.659	84	21-Dec	23-Dec	84	86	19
MD	2	1	5	15	1.423	5.9	7.323	113	19-Dec	21-Dec	82	84	15

MD	2	2	1	15	1.158	4.1	5.258	102	21-Dec	24-Dec	84	87	16
MD	2	2	2	15	1.112	4.8	5.912	103	24-Dec	25-Dec	87	88	19
MD	2	2	3	15	1.637	6.4	8.037	95	24-Dec	27-Dec	87	90	18
MD	2	2	4	15	1.182	4.4	5.582	104.5	24-Dec	27-Dec	87	90	15
MD	2	2	5	15	1.058	4.5	5.558	111	23-Dec	25-Dec	86	88	15
MD	2	3	1	18	1.689	5.3	6.989	100	4-Jan	6-Jan	98	100	13
MD	2	3	2	19	0.959	5	5.959	108	3-Jan	10-Jan	97	104	11
MD	2	3	3	19	0.757	3.5	4.257	97	7-Jan	12-Jan	101	106	15
MD	2	3	4	19	1.389	5.5	6.889	106	2-Jan	10-Jan	96	104	7
MD	2	3	5	18	0.775	4	4.775	103	5-Jan	6-Jan	99	100	10
MD	2	4	1	19	0.944	5.6	6.544	114	5-Jan	8-Jan	99	102	17
MD	2	4	2	19	1.832	6.6	8.432	103	5-Jan	7-Jan	99	101	28
MD	2	4	3	19	1.183	5.1	6.283	112	12-Jan	13-Jan	106	107	17
MD	2	4	4	19	0.867	5.3	6.167	107	11-Jan	15-Jan	105	109	6
MD	2	4	5	19	1.456	6.9	8.356	110	6-Jan	10-Jan	100	104	35
NC	1	1	1	18	0.702	4.4	5.102	106	4-Jan	5-Jan	98	99	18
NC	1	1	2	18	1.178	3.9	5.078	99	2-Jan	7-Jan	96	101	12
NC	1	1	3	19	1.294	4.9	6.194	95	14-Jan	17-Jan	108	111	26
NC	1	1	4	19	1.464	5.3	6.764	118	11-Jan	15-Jan	105	109	16
NC	1	1	5	19	1.157	4.5	5.657	126	13-Jan	14-Jan	107	108	18
NC	1	2	1	18	1.154	5.6	6.754	171	4-Jan	9-Jan	98	103	18
NC	1	2	2	19	1.432	5.3	6.732	115	12-Jan	14-Jan	106	108	17
NC	1	2	3	19	0.749	3.4	4.149	98	14-Jan	15-Jan	108	109	14
NC	1	2	4	19	0.539	1.1	1.639	71	3-Jan	5-Jan	97	99	8
NC	1	2	5	19	0.581	3.4	3.981	107	14-Jan	17-Jan	108	111	18
NC	1	3	1	21	1.555	5.8	7.355	140	29-Jan	31-Jan	123	125	10
NC	1	3	2	21	1.348	5.4	6.748	145	29-Jan	3-Feb	123	128	9
NC	1	3	3	19	1.057	4.8	5.857	121	29-Jan	1-Feb	123	126	12

NC	1	3	4	21	1.567	5.5	7.067	129	30-Jan	2-Feb	124	127	11
NC	1	3	5	21	1.016	4.8	5.816	115	28-Jan	29-Jan	122	123	8
NC	1	4	1	21	1.749	5.6	7.349	146	26-Jan	29-Jan	120	123	10
NC	1	4	2	22	1.994	6.9	8.894	141	17-Jan	22-Jan	111	116	19
NC	1	4	3	21	1.968	6.6	8.568	143	1-Feb	24-Feb	126	149	6
NC	1	4	4	21	1.523	8.2	9.723	144	2-Feb	5-Feb	127	130	13
NC	1	4	5	21	1.933	7.3	9.233	140	29-Jan	31-Jan	123	125	11
NC	2	1	1	19	1.685	5	6.685	118	9-Jan	12-Jan	103	106	13
NC	2	1	2	16	1.39	4.7	6.09	126	7-Jan	10-Jan	101	104	16
NC	2	1	3	19	1.683	5.2	6.883	111	9-Jan	12-Jan	103	106	17
NC	2	1	4	19	1.544	7.1	8.644	153	9-Jan	12-Jan	103	106	18
NC	2	1	5	19	1.132	4.5	5.632	105	3-Jan	7-Jan	97	101	11
NC	2	2	1	19	1.786	5.3	7.086	127	6-Jan	11-Jan	100	105	17
NC	2	2	2	19	1.355	4.5	5.855	107	12-Jan	13-Jan	106	107	15
NC	2	2	3	19	1.211	4.2	5.411	131	14-Jan	17-Jan	108	111	13
NC	2	2	4	19	1.48	5.6	7.08	145	2-Jan	5-Jan	96	99	20
NC	2	2	5	19	1.424	6.4	7.824	133	12-Jan	17-Jan	106	111	19
NC	2	3	1	19	1.427	5.5	6.927	143	29-Dec	31-Dec	92	94	12
NC	2	3	2	20	2.108	7.5	9.608	140	23-Jan	26-Jan	117	120	16
NC	2	3	3	21	1.689	6.9	8.589	154	13-Jan	16-Jan	107	110	13
NC	2	3	4	19	1.553	0	1.553	137	31-Jan	3-Feb	125	128	?
NC	2	3	5	21	1.455	5.8	7.255	134	2-Feb	4-Feb	127	129	11
NC	2	4	1	21	1.789	5.5	7.289	127	24-Jan	29-Jan	118	123	6
NC	2	4	2	21	2.053	7.8	9.853	157	24-Jan	24-Jan	118	118	14
NC	2	4	3	21	1.58	5.6	7.18	122	31-Jan	2-Feb	125	127	8
NC	2	4	4	21	1.978	6.2	8.178	119	29-Jan	1-Feb	123	126	16
NC	2	4	5	21	1.537	5.7	7.237	150	1-Feb	5-Feb	126	130	15
NJ	1	1	1	14	0.916	2.9	3.816	106	2-Dec	5-Dec	65	68	21

NJ	1	1	2	14	1.343	4.1	5.443	113	5-Dec	6-Dec	68	69	18
NJ	1	1	3	14	1.498	4.1	5.598	125.5	5-Dec	8-Dec	68	71	14
NJ	1	1	4	14	1.312	3.8	5.112	118	8-Dec	10-Dec	71	73	15
NJ	1	1	5	14	1.271	4.6	5.871	128	4-Dec	6-Dec	67	69	17
NJ	1	2	1	14	0.873	2.3	3.173	106	8-Dec	10-Dec	71	73	14
NJ	1	2	2	14	1.771	4.4	6.171	120	6-Dec	8-Dec	69	71	16
NJ	1	2	3	14	1.147	3.2	4.347	104	15-Dec	15-Dec	78	78	13
NJ	1	2	4	14	1.081	2.9	3.981	95	7-Dec	8-Dec	70	71	16
NJ	1	2	5	14	1.114	2.8	3.914	97	4-Dec	8-Dec	67	71	13
NJ	1	3	1	15	0.731	2.4	3.131	89	13-Dec	13-Dec	76	76	13
NJ	1	3	2	15	1.099	3.1	4.199	82.5	19-Dec	19-Dec	82	82	17
NJ	1	3	3	15	1.503	3.8	5.303	101	21-Dec	21-Dec	84	84	18
NJ	1	3	4	15	1.237	3.9	5.137	108	13-Dec	13-Dec	76	76	18
NJ	1	3	5	15	1.329	5.8	7.129	111	13-Dec	15-Dec	76	78	23
NJ	1	4	1	15	1.068	2.9	3.968	93	14-Dec	14-Dec	77	77	21
NJ	1	4	2	15	0.508	4.3	4.808	118	14-Dec	14-Dec	77	77	16
NJ	1	4	3	15	0.371	3.5	3.871	120	17-Dec	21-Dec	80	84	16
NJ	1	4	4	15	1.817	5.5	7.317	102	19-Dec	19-Dec	82	82	22
NJ	1	4	5	15	0.636	3.3	3.936	108	15-Dec	15-Dec	78	78	15
NJ	2	1	1	14	1.061	3.9	4.961	110	7-Dec	9-Dec	70	72	22
NJ	2	1	2	14	1.519	5.3	6.819	112	3-Dec	6-Dec	66	69	20
NJ	2	1	3	14	0.905	2.7	3.605	116	4-Dec	6-Dec	67	69	21
NJ	2	1	4	14	0.937	3.6	4.537	127	2-Dec	5-Dec	65	68	19
NJ	2	1	5	14	0.73	2.4	3.13	109	4-Dec	6-Dec	67	69	19
NJ	2	2	1	14	1.134	3.5	4.634	109	6-Dec	8-Dec	69	71	15
NJ	2	2	2	14	1.124	2.6	3.724	97	10-Dec	11-Dec	73	74	13
NJ	2	2	3	14	1.14	2.9	4.04	102	7-Dec	9-Dec	70	72	12
NJ	2	2	4	14	0.843	2.6	3.443	101	8-Dec	9-Dec	71	72	13

NJ	2	2	5	14	0.354	1.8	2.154	95	9-Dec	10-Dec	72	73	12
NJ	2	3	1	15	1.089	2.9	3.989	105	13-Dec	13-Dec	76	76	14
NJ	2	3	2	15	1.409	3.7	5.109	112	13-Dec	14-Dec	76	77	13
NJ	2	3	3	15	0.989	3.3	4.289	99	14-Dec	14-Dec	77	77	18
NJ	2	3	4	15	1.007	3.9	4.907	114	13-Dec	13-Dec	76	76	15
NJ	2	3	5	15	1.15	4	5.15	104	13-Dec	13-Dec	76	76	16
NJ	2	4	1	16	1.317	3.2	4.517	114	20-Dec	21-Dec	83	84	14
NJ	2	4	2	15	2.026	4.9	6.926	115	15-Dec	16-Dec	78	79	12
NJ	2	4	3	15	1.285	3.1	4.385	107	19-Dec	22-Dec	82	85	16
NJ	2	4	4	15	0.98	4.2	5.18	88	15-Dec	16-Dec	78	79	16
NJ	2	4	5	15	1.058	3	4.058	106	13-Dec	14-Dec	76	77	15
PA	1	1	1	14	0.757	3.2	3.957	115	2-Dec	5-Dec	65	68	14
PA	1	1	2	14	0.766	2	2.766	89	5-Dec	7-Dec	68	70	7
PA	1	1	3	14	1.005	2.9	3.905	95.5	4-Dec	6-Dec	67	69	10
PA	1	1	4	14	1.229	5.2	6.429	112	7-Dec	9-Dec	70	72	15
PA	1	1	5	14	1.605	4	5.605	121	7-Dec	9-Dec	70	72	11
PA	1	2	1	14	1.088	3.6	4.688	100	3-Dec	5-Dec	66	68	11
PA	1	2	2	14	1.235	4.1	5.335	97	8-Dec	10-Dec	71	73	16
PA	1	2	3	14	0.925	2.8	3.725	103	6-Dec	9-Dec	69	72	6
PA	1	2	4	14	0.536	1.6	2.136	93	6-Dec	8-Dec	69	71	4
PA	1	2	5	14	1.079	3.7	4.779	120	4-Dec	6-Dec	67	69	8
PA	1	3	1	16	0.737	2.7	3.437	90	19-Dec	21-Dec	82	84	10
PA	1	3	2	15	2.041	6.4	8.441	128	15-Dec	16-Dec	78	79	11
PA	1	3	3	15	0.757	3.7	4.457	113	24-Dec	24-Dec	87	87	14
PA	1	3	4	15	1.009	4	5.009	104	24-Dec	24-Dec	87	87	14
PA	1	3	5	15	0.941	4.4	5.341	112.5	16-Dec	19-Dec	79	82	12
PA	1	4	1	16	1.075	3.5	4.575	103	22-Dec	23-Dec	85	86	13
PA	1	4	2	15	1.085	3.1	4.185	145	19-Dec	21-Dec	82	84	18

PA	1	4	3	15	0.691	4.5	5.191	98.5	21-Dec	22-Dec	84	85	20
PA	1	4	4	16	1.972	6.5	8.472	106	27-Dec	27-Dec	90	90	14
PA	1	4	5	15	0.858	2.7	3.558	92	19-Dec	19-Dec	82	82	17
PA	2	1	1	14	0.882	2.4	3.282	94	30-Nov	1-Dec	63	64	23
PA	2	1	2	14	0.72	1.9	2.62	104	2-Dec	4-Dec	65	67	7
PA	2	1	3	14	0.917	3.7	4.617	104	8-Dec	10-Dec	71	73	21
PA	2	1	4	14	0.986	3	3.986	107	2-Dec	5-Dec	65	68	13
PA	2	1	5	14	0.867	2.7	3.567	101	7-Dec	8-Dec	70	71	17
PA	2	2	1	14	0.769	2.4	3.169	113	7-Dec	8-Dec	70	71	6
PA	2	2	2	14	0.57	1.7	2.27	85.5	7-Dec	9-Dec	70	72	6
PA	2	2	3	14	0.651	1.8	2.451	94	5-Dec	7-Dec	68	70	8
PA	2	2	4	14	0.735	1.7	2.435	93	6-Dec	8-Dec	69	71	12
PA	2	2	5	14	0.603	1.9	2.503	93	7-Dec	9-Dec	70	72	10
PA	2	3	1	16	1.56	4.9	6.46	112	27-Dec	28-Dec	90	91	6
PA	2	3	2	15	1.014	4.2	5.214	98	21-Dec	21-Dec	84	84	10
PA	2	3	3	15	0.943	3.8	4.743	97.5	21-Dec	22-Dec	84	85	13
PA	2	3	4	16	0.841	4	4.841	117	24-Dec	24-Dec	87	87	9
PA	2	3	5	15	0.831	5	5.831	121	14-Dec	16-Dec	77	79	8
PA	2	4	1	16	1.535	3.8	5.335	101	24-Dec	26-Dec	87	89	15
PA	2	4	2	16	1.759	4.1	5.859	107	22-Dec	23-Dec	85	86	8
PA	2	4	3	15	1.633	4.9	6.533	105	21-Dec	22-Dec	84	85	23
PA	2	4	4	16	1.548	5.2	6.748	93	30-Dec	2-Jan	93	96	7
PA	2	4	5	16	0.586	2.9	3.486	97	20-Dec	21-Dec	83	84	18
SC	1	1	1	16	1.218	4.3	5.518	116	7-Jan	9-Jan	101	103	20
SC	1	1	2	18	1.958	4.9	6.858	112	8-Jan	9-Jan	102	103	7
SC	1	1	3	18	2.378	5.4	7.778	121	5-Jan	6-Jan	99	100	12
SC	1	1	4	19	1.633	4.5	6.133	97	5-Jan	6-Jan	99	100	6
SC	1	1	5	16	1.167	4	5.167	100	5-Jan	6-Jan	99	100	6

SC	1	2	1	19	1.653	4.4	6.053	134	9-Jan	9-Jan	103	103	7
SC	1	2	2	18	2.332	6.3	8.632	126	4-Jan	6-Jan	98	100	12
SC	1	2	3	19	2.422	4.9	7.322	108	7-Jan	10-Jan	101	104	10
SC	1	2	4	19	1.818	4.2	6.018	112	7-Jan	10-Jan	101	104	17
SC	1	2	5	16	1.084	3.4	4.484	96	9-Jan	10-Jan	103	104	10
SC	1	3	1	20	2.364	5.9	8.264	137	27-Jan	29-Jan	121	123	8
SC	1	3	2	20	2.57	6.1	8.67	138	15-Jan	15-Jan	109	109	11
SC	1	3	3	20	1.744	4.9	6.644	113	23-Jan	24-Jan	117	118	11
SC	1	3	4	20	1.789	4.1	5.889	135	25-Jan	29-Jan	119	123	6
SC	1	3	5	21	1.628	5.5	7.128	125	25-Jan	27-Jan	119	121	12
SC	1	4	1	21	2.192	6.1	8.292	134	27-Jan	30-Jan	121	124	10
SC	1	4	2	21	2.879	6.3	9.179	108	29-Jan	30-Jan	123	124	15
SC	1	4	3	21	2.481	5.4	7.881	128	28-Jan	30-Jan	122	124	8
SC	1	4	4	21	2.186	6	8.186	133	26-Jan	28-Jan	120	122	15
SC	1	4	5	21	2.332	7.4	9.732	124	20-Jan	21-Jan	114	115	17
SC	2	1	1	16	1.485	4.8	6.285	118	7-Jan	8-Jan	101	102	9
SC	2	1	2	19	1.51	4.4	5.91	114	7-Jan	8-Jan	101	102	6
SC	2	1	3	19	1.124	3.9	5.024	122	4-Jan	6-Jan	98	100	14
SC	2	1	4	19	1.596	4.2	5.796	101	11-Jan	12-Jan	105	106	15
SC	2	1	5	18	1.317	3.5	4.817	104	10-Jan	10-Jan	104	104	10
SC	2	2	1	19	1.888	5.2	7.088	136	6-Jan	10-Jan	100	104	8
SC	2	2	2	19	2.459	6.9	9.359	125	5-Jan	7-Jan	99	101	17
SC	2	2	3	19	1.691	4.3	5.991	129	8-Jan	10-Jan	102	104	9
SC	2	2	4	19	0.956	2.8	3.756	88	11-Jan	12-Jan	105	106	12
SC	2	2	5	16	2.118	7.3	9.418	138	7-Jan	10-Jan	101	104	11
SC	2	3	1	20	1.806	6	7.806	138	13-Jan	14-Jan	107	108	12
SC	2	3	2	20	2.037	5.1	7.137	133	25-Jan	31-Jan	119	125	10
SC	2	3	3	20	3.094	7.9	10.994	124	22-Jan	25-Jan	116	119	14

SC	2	3	4	21	1.813	5.1	6.913	138	26-Jan	28-Jan	120	122	7
SC	2	3	5	20	1.936	5.8	7.736	113	24-Jan	26-Jan	118	120	14
SC	2	4	1	20	1.807	4.5	6.307	147	27-Jan	28-Jan	121	122	8
SC	2	4	2	20	2.152	5.7	7.852	132	25-Jan	28-Jan	119	122	6
SC	2	4	3	21	2.794	5	7.794	100	26-Jan	31-Jan	120	125	9
SC	2	4	5	21	1.871	5.5	7.371	126	10-Jan	11-Jan	104	105	17
VA1	1	1	1	15	0.826	4.1	4.926	136	21-Dec	25-Dec	84	88	12
VA1	1	1	2	16	0.804	3.2	4.004	104	30-Dec	1-Jan	93	95	10
VA1	1	1	3	16	1.646	4.8	6.446	114	29-Dec	1-Jan	92	95	8
VA1	1	1	4	16	1.047	3.3	4.347	95	30-Dec	31-Dec	93	94	6
VA1	1	1	5	16	1.325	4.4	5.725	125	31-Dec	2-Jan	94	96	6
VA1	1	2	1	16	0.984	3.5	4.484	126	26-Dec	28-Dec	89	91	7
VA1	1	2	2	16	1.296	4	5.296	108	30-Dec	31-Dec	93	94	6
VA1	1	2	3	16	1.202	5	6.202	110	30-Dec	31-Dec	93	94	9
VA1	1	2	4	18	1.705	5.1	6.805	108	30-Dec	1-Jan	93	95	8
VA1	1	2	5	16	0.709	4.2	4.909	117.5	25-Dec	26-Dec	88	89	5
VA1	1	3	1	19	1.139	4.1	5.239	97	6-Jan	10-Jan	100	104	14
VA1	1	3	1	19	1.694	5.8	7.494	102	8-Jan	8-Jan	102	102	11
VA1	1	3	2	19	1.895	5.6	7.495	109	7-Jan	10-Jan	101	104	10
VA1	1	3	3	18	1.497	4.2	5.697	111	6-Jan	7-Jan	100	101	7
VA1	1	3	4	19	1.313	5.2	6.513	121	5-Jan	6-Jan	99	100	12
VA1	2	3	5	16	1.164	4.9	6.064	104	1-Jan	4-Jan	95	98	16
VA1	1	4	1	19	1.841	5.6	7.441	119	14-Jan	16-Jan	108	110	8
VA1	1	4	2	20	1.559	4.6	6.159	104	14-Jan	15-Jan	108	109	6
VA1	1	4	3	20	1.703	4.7	6.403	103	16-Jan	17-Jan	110	111	6
VA1	1	4	4	20	1.392	5.8	7.192	110	17-Jan	18-Jan	111	112	15
VA1	1	4	5	19	2.474	6.7	9.174	116	7-Jan	9-Jan	101	103	12
VA1	2	1	1	16	1.768	5.8	7.568	139	25-Dec	27-Dec	88	90	9

VA1	2	1	2	16	1.234	4.1	5.334	120	27-Dec	27-Dec	90	90	10
VA1	2	1	3	16	1.084	4.5	5.584	124	29-Dec	31-Dec	92	94	11
VA1	2	1	4	16	1.844	5.4	7.244	120	29-Dec	Dec-32	92		8
VA1	2	1	5	18	1.72	5.6	7.32	126	29-Dec	31-Dec	92	94	9
VA1	2	2	1	16	1.247	3.6	4.847	102	28-Dec	29-Dec	91	92	5
VA1	2	2	2	16	1.084	4.2	5.284	110	28-Dec	30-Dec	91	93	5
VA1	2	2	3	15	1.66	5.8	7.46	95	1-Jan	2-Jan	95	96	12
VA1	2	2	4	16	1.001	2.9	3.901	94.5	28-Dec	30-Dec	91	93	7
VA1	2	2	5	16	0.84	3.3	4.14	102	30-Dec	1-Jan	93	95	6
VA1	2	3	2	19	1.77	5.7	7.47	123	30-Dec	Dec-10	93	94	10
VA1	2	3	3	19	2.478	7.6	10.078	134	13-Jan	14-Jan	107	108	13
VA1	2	3	4	16	1.105	6.2	7.305	122	31-Dec	3-Jan	94	97	14
VA1	2	3	5	18	1.355	4.6	5.955	119	1-Jan	2-Jan	95	96	16
VA1	2	4	1	19	1.259	4	5.259	126	7-Jan	9-Jan	101	103	6
VA1	2	4	2	20	1.644	5	6.644	107	9-Jan	9-Jan	103	103	5
VA1	2	4	3	19	1.582	5.7	7.282	99	18-Jan	19-Jan	112	113	14
VA1	2	4	4	18	2.138	8.2	10.338	121	6-Jan	7-Jan	100	101	13
VA1	2	4	5	19	2.018	7	9.018	112	12-Jan	13-Jan	106	107	9
VA2	1	1	1	16	0.896	3.2	4.096	119	1-Jan	5-Jan	95	99	10
VA2	1	1	2	15	0.648	4.4	5.048	98	25-Dec	28-Dec	88	91	11
VA2	1	1	3	16	1.998	5	6.998	129	2-Jan	4-Jan	96	98	11
VA2	1	1	4	14	1.096	4	5.096	85.5	15-Dec	21-Dec	78	84	18
VA2	1	1	5	16	1.821	4.6	6.421	106	2-Jan	4-Jan	96	98	11
VA2	1	2	1	14	1.962	4.8	6.762	117	14-Dec	19-Dec	77	82	15
VA2	1	2	2	14	0.928	2.8	3.728	78	11-Dec	15-Dec	74	78	14
VA2	1	2	3	16	1.871	4.9	6.771	116	3-Jan	5-Jan	97	99	12
VA2	1	2	4	15	0.602	4.2	4.802	97	28-Dec	30-Dec	91	93	16
VA2	1	2	5	16	1.529	3.6	5.129	107	25-Dec	30-Dec	88	93	10

VA2	1	3	1	18	1.758	6.8	8.558	124	6-Jan	6-Jan	100	100	15
VA2	1	3	2	16	2.266	5.4	7.666	?	6-Jan	10-Jan	100	104	11
VA2	1	3	3	16	1.508	5.7	7.208	118	30-Dec	1-Jan	93	95	7
VA2	1	3	4	16	0.623	4.2	4.823	92	31-Dec	1-Jan	94	95	32
VA2	1	3	5	16	0.915	4.6	5.515	107	2-Jan	4-Jan	96	98	18
VA2	1	4	1	18	1.511	5	6.511	139	9-Jan	12-Jan	103	106	6
VA2	1	4	2	16	1.838	4.7	6.538	96	27-Dec	30-Dec	90	93	9
VA2	1	4	3	16	1.811	4.4	6.211	109	29-Dec	31-Dec	92	94	14
VA2	1	4	4	16	1.979	5.9	7.879	131	29-Dec	2-Jan	92	96	9
VA2	1	4	5	20	2.025	5.6	7.625	134	18-Jan	23-Jan	112	117	13
VA2	2	1	1	16	1.553	7	8.553	111	20-Dec	21-Dec	83	84	15
VA2	2	1	2	15	1.417	4.3	5.717	135	21-Dec	21-Dec	84	84	?
VA2	2	1	3	14	0.944	3.2	4.144	103	7-Dec	9-Dec	70	72	23
VA2	2	1	4	14	1.018	3	4.018	100	7-Dec	10-Dec	70	73	13
VA2	2	1	5	14	0.663	2.6	3.263	113	6-Dec	8-Dec	69	71	15
VA2	2	2	1	14	1.339	3.3	4.639	105	16-Dec	17-Dec	79	80	13
VA2	2	2	2	15	0.806	2.5	3.306	100	17-Dec	20-Dec	80	83	7
VA2	2	2	3	14	0.788	2.2	2.988	76	7-Dec	10-Dec	70	73	12
VA2	2	2	4	15	0.487	2.6	3.087	100	20-Dec	22-Dec	83	85	9
VA2	2	2	5	14	0.698	2	2.698	86	7-Dec	10-Dec	70	73	16
VA2	2	3	1	18	0.834	3.5	4.334	106	2-Jan	4-Jan	96	98	9
VA2	2	3	2	19	1.373	5.3	6.673	93	2-Jan	5-Jan	96	99	29
VA2	2	3	3	16	1.427	5.1	6.527	132	5-Jan	6-Jan	99	100	16
VA2	2	3	4	16	1.143	5.3	6.443	101	23-Dec	26-Dec	86	89	10
VA2	2	3	5	16	2.138	5.7	7.838	107	22-Dec	24-Dec	85	87	14
VA2	2	4	1	20	1.621	3.6	5.221	109	15-Jan	16-Jan	109	110	11
VA2	2	4	2	20	1.234	4.5	5.734	109	15-Jan	15-Jan	109	109	9
VA2	2	4	3	19	1.158	6	7.158	117	9-Jan	11-Jan	103	105	27

VA2	2	4	4	16	2.222	5.9	8.122	81	27-Dec	30-Dec	90	93	23
VA2	2	4	5	18	1.672	5.8	7.472	128	30-Dec	1-Jan	93	95	12
WV	1	1	1	14	0.706	2.8	3.506	86	7-Dec	9-Dec	70	72	25
WV	1	1	2	14	1.278	4	5.278	118	30-Nov	2-Dec	63	65	15
WV	1	1	3	16	0.635	4.3	4.935	84	23-Dec	26-Dec	86	89	9
WV	1	1	4	15	0.667	3.7	4.367	95	19-Dec	23-Dec	82	86	12
WV	1	1	5	15	0.89	3.6	4.49	105	17-Dec	21-Dec	80	84	11
WV	1	2	1	14	0.771	2.4	3.171	75	2-Dec	4-Dec	65	67	10
WV	1	2	2	15	0.726	2.6	3.326	98	19-Dec	20-Dec	82	83	7
WV	1	2	3	14	1.169	3.9	5.069	81	4-Dec	7-Dec	67	70	20
WV	1	2	4	14	0.812	2.8	3.612	90	11-Dec	13-Dec	74	76	18
WV	1	2	5	14	1.33	4.6	5.93	106	9-Dec	11-Dec	72	74	14
WV	1	3	1	19	0.899	3.6	4.499	90	4-Jan	7-Jan	98	101	7
WV	1	3	2	19	0.866	4.6	5.466	123	10-Jan	10-Jan	104	104	16
WV	1	3	3	15	0.946	3.7	4.646	95	13-Dec	13-Dec	76	76	17
WV	1	3	4	16	1.098	3.7	4.798	101	15-Dec	19-Dec	78	82	14
WV	1	3	5	15	1.069	4.8	5.869	110	17-Dec	21-Dec	80	84	12
WV	1	4	1	16	0.848	2.9	3.748	111	22-Dec	23-Dec	85	86	10
WV	1	4	2	19	0.763	4.5	5.263	94	12-Jan	14-Jan	106	108	9
WV	1	4	3	16	1.398	4	5.398	104	19-Dec	22-Dec	82	85	16
WV	1	4	4	15	1.68	6.1	7.78	96	19-Dec	21-Dec	82	84	25
WV	1	4	5	16	1.57	5.9	7.47	106	19-Dec	19-Dec	82	82	25
WV	2	1	1	14	1.813	4.4	6.213	107	1-Dec	4-Dec	64	67	21
WV	2	1	2	14	1.032	4	5.032	106	7-Dec	9-Dec	70	72	19
WV	2	1	3	14	1.146	4.2	5.346	106	13-Dec	14-Dec	76	77	16
WV	2	1	4	16	0.503	2.8	3.303	102	21-Dec	23-Dec	84	86	9
WV	2	1	5	15	0.84	4.3	5.14	117	15-Dec	19-Dec	78	82	10
WV	2	2	1	14	1.124	3.6	4.724	118	3-Dec	5-Dec	66	68	15

WV	2	2	2	14	1.029	3.3	4.329	107	7-Dec	11-Dec	70	74	13
WV	2	2	3	15	0.806	3.8	4.606	106	21-Dec	23-Dec	84	86	11
WV	2	2	4	14	0.733	2.6	3.333	95	5-Dec	6-Dec	68	69	14
WV	2	2	5	14	1.133	3.5	4.633	97	2-Dec	3-Dec	65	66	12
WV	2	3	1	16	1.073	3.7	4.773	107.5	16-Dec	19-Dec	79	82	19
WV	2	3	2	15	1.238	4.3	5.538	95	19-Dec	21-Dec	82	84	11
WV	2	3	3	19	1.208	4.8	6.008	125	4-Jan	10-Jan	98	104	11
WV	2	3	4	19	0.502	2.2	2.702	94	10-Jan	12-Jan	104	106	8
WV	2	3	5	15	0.94	4.9	5.84	113.5	19-Dec	21-Dec	82	84	21
WV	2	4	1	15	0.249	3.2	3.449	88	17-Dec	21-Dec	80	84	15
WV	2	4	2	15	0.609	3.1	3.709	78	13-Dec	16-Dec	76	79	13
WV	2	4	3	16	1.376	4.2	5.576	92	21-Dec	23-Dec	84	86	14
WV	2	4	4	19	1.221	6.2	7.421	107	9-Jan	11-Jan	103	105	17
WV	2	4	5	15	1.536	4.4	5.936	109	19-Dec	21-Dec	82	84	16

Table B.5. Raw data of Asian sourced plants used in the growth chamber experiment sorted by population, rep, chamber, and block. Senescence, inflorescence day (i.e., day of first emergence of immature inflorescence), and flowering day (i.e., day of first anthesis) are measured in number of days or weeks since seeds were germinated. Biomasses, height and terminal inflorescence counts were measured at senescence. GPS coordinates for each population are presented in Table B.1. Dates are in the winter of 2010-11. Chamber 1 and 2 are the southern light treatment. Chamber 3 and 4 are the northern light treatment. Populations CN2, CN6, and CN9 were removed from the dataset because they were contaminants of different species of *Microstegium* or *Arthraxon*.

Pop	Rep	Chamber	Block	Senescence (wk)	Root Biomass (g)	Aerial Biomass (g)	Total Biomass (g)	Final Height (cm)	Infl. Date	Flowering Date	Infl. Day	Flowering Day	# Term Infl.
CN1	1	1	1	22	1.831	5.2	7.031	127	25-Jan	29-Jan	119	123	19
CN1	1	1	3	22	2.147	6.5	8.647	137	27-Jan	31-Jan	121	125	18
CN1	1	1	5	21	1.803	5.1	6.903	116	1-Feb	7-Feb	126	132	17
CN1	1	2	1	21	2.244	7.6	9.844	161	27-Jan	31-Jan	121	125	31
CN1	1	2	2	21	2.292	8.1	10.392	139	23-Jan	27-Jan	117	121	16
CN1	1	2	3	21	1.226	5.2	6.426	132	26-Jan	29-Jan	120	123	22
CN1	1	2	4	21	0.471	3	3.471	93	29-Jan	2-Feb	123	127	17
CN1	1	2	5	21	2.009	6.7	8.709	119	27-Jan	29-Jan	121	123	17
CN1	1	3	1	22	1.809	6.3	8.109	140	27-Jan	2-Feb	121	127	12
CN1	1	3	3	22	1.359	5.3	6.659	159	5-Feb	5-Feb	130	130	13
CN1	1	3	4	22	1.81	8.6	10.41	166	3-Feb	7-Feb	128	132	18
CN1	1	3	5	22	1.535	6.9	8.435	156	5-Feb	7-Feb	130	132	16
CN1	1	4	1	22	1.871	5.9	7.771	139	29-Jan	3-Feb	123	128	11
CN1	1	4	2	25	1.279	6.5	7.779	159	20-Feb	27-Feb	145	152	30
CN1	1	4	3	22	1.474	5.4	6.874	127	5-Feb	8-Feb	130	133	28
CN1	1	4	4	22	2.096	7.6	9.696	133	2-Feb	5-Feb	127	130	21
CN1	1	4	5	22	1.535	8.3	9.835	147	5-Feb	7-Feb	130	132	31
CN1	2	1	2	21	1.191	5.2	6.391	130	28-Jan	29-Jan	122	123	16

CN1	2	1	3	22	2.166	7.5	9.666	140	25-Jan	29-Jan	119	123	16
CN1	2	1	4	21	2.131	7.8	9.931	133	28-Jan	1-Feb	122	126	30
CN1	2	1	5	21	0.839	3.6	4.439	108	28-Jan	1-Feb	122	126	16
CN1	2	2	1	22	1.952	6.5	8.452	132	24-Jan	29-Jan	118	123	17
CN1	2	2	2	21	1.74	5.8	7.54	129	25-Jan	31-Jan	119	125	18
CN1	2	2	3	21	2.336	8.2	10.536	114	27-Jan	1-Feb	121	126	22
CN1	2	2	4	21	2.115	7.8	9.915	147	27-Jan	29-Jan	121	123	16
CN1	2	2	5	24	1.195	7.3	8.495	152	12-Feb	17-Feb	137	142	16
CN1	2	3	1	22	2.049	9.1	11.149	162	29-Jan	5-Feb	123	130	19
CN1	2	3	2	22	1.968	7.6	9.568	130	27-Jan	30-Jan	121	124	21
CN1	2	3	3	22	1.791	9	10.791	167	5-Feb	8-Feb	130	133	16
CN1	2	3	4	22	2.174	8.3	10.474	129	31-Jan	3-Feb	125	128	18
CN1	2	3	5	22	1.734	7.2	8.934	146	1-Feb	5-Feb	126	130	12
CN1	2	4	1	22	1.194	4.9	6.094	141	3-Feb	8-Feb	128	133	15
CN1	2	4	4	22	1.99	8.6	10.59	141	5-Feb	6-Feb	130	131	24
CN1	2	4	5	22	2.614	11.4	14.014	128	5-Feb	7-Feb	130	132	34
CN3	1	1	1	23	3.231	9.5	12.731	137	12-Feb	15-Feb	137	140	28
CN3		1	2	24	2.345	6.1	8.445	122	13-Feb	19-Feb	138	144	21
CN3	1	1	3	23	3.146	10.3	13.446	136	8-Feb	12-Feb	133	137	36
CN3	1	1	4	23	3.327	9.9	13.227	137	8-Feb	14-Feb	133	139	17
CN3	1	2	1	23	2.285	8.7	10.985	138	7-Feb	13-Feb	132	138	25
CN3	1	2	3	25	2.021	4.8	6.821	121	7-Feb	11-Feb	132	136	12
CN3	1	2	4	24	2.352	7.5	9.852	126	11-Feb	17-Feb	136	142	16
CN3	1	2	5	24	1.425	5.3	6.725	147	10-Feb	17-Feb	135	142	12
CN3	1	3	1	24	1.953	7.3	9.253	148	15-Feb	21-Feb	140	146	21
CN3	1	3	3	25	2.136	6.5	8.636	153	21-Feb	25-Feb	146	150	15
CN3	1	3	5	25	2.031	6.7	8.731	162	21-Feb	25-Feb	146	150	12
CN3	1	4	2	27	2.659	8.1	10.759	140	22-Feb	27-Feb	147	152	23

CN3	1	4	3	26	1.854	7.3	9.154	137	18-Feb	25-Feb	143	150	25
CN3	1	4	4	25	3.128	9	12.128	124	19-Feb	23-Feb	144	148	44
CN3	1	4	5	25	2.346	9.3	11.646	137	20-Feb	25-Feb	145	150	18
CN3	2	1	1	23	2.887	8.5	11.387	133	9-Feb	13-Feb	134	138	22
CN3	2	1	2	24	2.539	7.2	9.739	124	12-Feb	17-Feb	137	142	13
CN3	2	1	3	23	3.993	9	12.993	131	7-Feb	13-Feb	132	138	20
CN3	2	1	4	23	3.564	9.7	13.264	146	8-Feb	13-Feb	133	138	21
CN3	2	1	5	24	3.17	8.7	11.87	135	8-Feb	11-Feb	133	136	21
CN3	2	2	1	24	1.847	4.6	6.447	153	8-Feb	16-Feb	133	141	7
CN3	2	2	2	25	1.639	5.8	7.439	92	16-Feb	28-Feb	141	153	28
CN3	2	2	3	24	2.269	5.8	8.069	131	11-Feb	14-Feb	136	139	21
CN3	2	2	4	24	2.076	7.6	9.676	155	11-Feb	18-Feb	136	143	16
CN3	2	2	5	24	2.603	8.7	11.303	154	9-Feb	16-Feb	134	141	28
CN3	2	3	3	25	1.142	6	7.142	130	21-Feb	25-Feb	146	150	14
CN3	2	4	2	25	1.688	6.5	8.188	135	26-Feb	2-Mar	151	155	36
CN3	2	4	3	24	3.039	0	3.039	154	19-Feb	22-Feb	144	147	30
CN4	1	1	1	23	2.351	7.7	10.051	142	12-Feb	18-Feb	137	143	11
CN4	1	1	2	24	3.302	8.7	12.002	121	12-Feb	16-Feb	137	141	25
CN4	1	1	3	22	2.14	7.1	9.24	141	6-Feb	12-Feb	131	137	26
CN4	1	1	4	23	1.841	7.8	9.641	171	3-Feb	8-Feb	128	133	14
CN4	1	1	5	25	2.763	8.5	11.263	131	10-Feb	14-Feb	135	139	31
CN4	1	2	1	24	2.719	9.2	11.919	147	10-Feb	15-Feb	135	140	14
CN4	1	2	2	24	2.236	6	8.236	148	11-Feb	16-Feb	136	141	13
CN4	1	2	3	24	2.696	7.7	10.396	162	11-Feb	15-Feb	136	140	17
CN4	1	2	4	23	2.045	5.6	7.645	138	13-Feb	18-Feb	138	143	8
CN4	1	2	5	23	2.726	10	12.726	159	10-Feb	15-Feb	135	140	18
CN4	1	3	1	25	1.446	5.8	7.246	127	14-Feb	19-Feb	139	144	9
CN4	1	3	2	25	2.71	8.4	11.11	127	19-Feb	26-Feb	144	151	16

CN4	1	3	4	25	1.526	5.8	7.326	121	22-Feb	27-Feb	147	152	13
CN4	1	3	5	25	2.251	7.9	10.151	167	21-Feb	25-Feb	146	150	10
CN4	1	4	1	25	2.869	8.8	11.669	143	19-Feb	24-Feb	144	149	16
CN4	1	4	2	25	1.869	7.4	9.269	144	22-Feb	28-Feb	147	153	15
CN4	1	4	4	25	2.166	7.6	9.766	134	19-Feb	24-Feb	144	149	20
CN4	1	4	5	23	1.653	6.9	8.553	144	5-Feb	8-Feb	130	133	21
CN4	2	1	1	23	2.79	7.1	9.89	141	9-Feb	13-Feb	134	138	18
CN4	2	1	2	24	3.182	10.4	13.582	160	2-Feb	2-Feb	127	127	22
CN4	2	1	3	23	3.187	11.3	14.487	133	11-Feb	16-Feb	136	141	27
CN4	2	1	5	24	3.06	8.6	11.66	125	9-Feb	15-Feb	134	140	39
CN4	2	2	1	24	2.47	7.4	9.87	119	12-Feb	19-Feb	137	144	37
CN4	2	2	2	24	3.304	9.5	12.804	113	11-Feb	19-Feb	136	144	22
CN4	2	2	3	24	2.322	6.9	9.222	139	12-Feb	16-Feb	137	141	16
CN4	2	2	4	24	2.693	7.9	10.593	129	11-Feb	17-Feb	136	142	16
CN4	2	2	5	25	2.559	7.2	9.759	138	12-Feb	16-Feb	137	141	14
CN4	2	3	1	25	2.111	8.2	10.311	148	18-Feb	24-Feb	143	149	16
CN4	2	3	2	25	2.451	9.7	12.151	163	19-Feb	23-Feb	144	148	13
CN4	2	3	3	24	2.214	7.1	9.314	143	18-Feb	23-Feb	143	148	13
CN4	2	3	4	25	1.628	6.2	7.828	144	20-Feb	25-Feb	145	150	16
CN4	2	4	1	25	2.03	7	9.03	150	19-Feb	25-Feb	144	150	10
CN4	2	4	3	25	2.542	7.6	10.142	122	18-Feb	24-Feb	143	149	18
CN4	2	4	4	24	1.967	7.8	9.767	117	8-Feb	21-Feb	133	146	21
CN4	2	4	5	24	2.997	8.4	11.397	145	19-Feb	23-Feb	144	148	17
CN5	1	1	1	25	2.445	8.1	10.545	156	11-Feb	19-Feb	136	144	17
CN5	1	1	2	25	2.109	7.6	9.709	174	15-Feb	18-Feb	140	143	45
CN5	1	1	3	24	1.399	9.1	10.499	143	6-Feb	9-Feb	131	134	33
CN5	1	1	5	24	3.291	12.1	15.391	153	15-Feb	19-Feb	140	144	38
CN5	1	2	1	25	1.934	6.7	8.634	172	17-Feb	19-Feb	142	144	13

CN5	1	2	2	24	2.276	7.2	9.476	144	18-Feb	21-Feb	143	146	17
CN5	1	2	3	25	2.041	6.7	8.741	130	15-Feb	20-Feb	140	145	22
CN5	1	2	4	25	1.949	8.6	10.549	176	21-Feb	28-Feb	146	153	32
CN5	1	2	5	24	1.721	7.9	9.621	162	17-Feb	21-Feb	142	146	16
CN5	1	3	1	26	1.626	7.5	9.126	126	26-Feb	2-Mar	151	155	19
CN5	1	3	2	25	2.303	9.3	11.603	152	25-Feb	28-Feb	150	153	18
CN5	1	3	3	25	1.89	7.4	9.29	172	19-Feb	22-Feb	144	147	21
CN5	1	3	5	25	1.744	5.8	7.544	152	24-Feb	2-Mar	149	155	15
CN5	1	4	1	25	1.522	6.6	8.122	130	26-Feb	2-Mar	151	155	21
CN5	1	4	2	26	2.252	9.6	11.852	161	22-Feb	25-Feb	147	150	11
CN5	1	4	4	25	3.016	11.6	14.616	195	24-Feb	25-Feb	149	150	18
CN5	2	1	1	26	2.142	8.9	11.042	144	18-Feb	21-Feb	143	146	25
CN5	2	1	2	25	2.001	7.3	9.301	133	19-Feb	21-Feb	144	146	19
CN5	2	1	3	24	2.69	9.1	11.79	132	5-Feb	19-Feb	130	144	23
CN5	2	1	4	24	2.753	9.7	12.453	146	13-Feb	15-Feb	138	140	22
CN5	2	2	1	25	1.661	4.9	6.561	172	17-Feb	21-Feb	142	146	12
CN5	2	2	3	25	2.003	6.9	8.903	167	24-Feb	28-Feb	149	153	30
CN5	2	2	4	25	1.935	8.5	10.435	161	22-Feb	2-Mar	147	155	25
CN5	2	2	5	25	1.415	5.1	6.515	141	19-Feb	21-Feb	144	146	16
CN5	2	3	2	26	2.191	9.4	11.591	157	25-Feb	27-Feb	150	152	21
CN5	2	3	3	25	1.107	6.1	7.207	130	27-Feb	1-Mar	152	154	17
CN5	2	3	4	25	1.502	6.4	7.902	146	19-Feb	22-Feb	144	147	17
CN5	2	3	5	25	1.647	7	8.647	148	19-Feb	24-Feb	144	149	17
CN5	2	4	1	25	2.368	8.3	10.668	140	24-Feb	28-Feb	149	153	24
CN5	2	4	2	24	1.407	7	8.407	144	19-Feb	24-Feb	144	149	18
CN5	2	4	3	25	2.066	7.1	9.166	160	24-Feb	26-Feb	149	151	14
CN5	2	4	4	25	2.575	12.6	15.175	158	19-Feb	22-Feb	144	147	34
CN5	2	4	5	26	2.261	8.6	10.861	144	24-Feb	1-Mar	149	154	16

CN7	1	1	1	23	1.476	3.9	5.376	118	3-Feb	7-Feb	128	132	20
CN7	1	1	3	23	1.72	7.7	9.42	110	4-Feb	7-Feb	129	132	28
CN7	1	1	4	21	1.948	6.9	8.848	168	28-Jan	2-Feb	122	127	8
CN7	1	1	5	24	2.694	7.8	10.494	120	5-Feb	6-Feb	130	131	15
CN7	1	2	1	23	2.183	6.8	8.983	157	3-Feb	9-Feb	128	134	35
CN7	1	2	2	22	1.806	4.8	6.606	114	27-Jan	1-Feb	121	126	7
CN7	1	2	3	22	1.563	4.8	6.363	150	26-Jan	27-Jan	120	121	17
CN7	1	2	4	23	1.14	3.9	5.04	127	5-Feb	10-Feb	130	135	19
CN7	1	2	5	22	1.598	4.4	5.998	127	2-Feb	4-Feb	127	129	19
CN7	1	3	3	25	1.598	6.4	7.998	138	15-Feb	21-Feb	140	146	26
CN7	1	3	4	25	1.895	6.2	8.095	156	15-Feb	19-Feb	140	144	29
CN7	1	3	5	25	1.002	6	7.002	146	12-Feb	18-Feb	137	143	8
CN7	1	4	2	25	2.46	6.7	9.16	152	13-Feb	16-Feb	138	141	37
CN7	1	4	3	25	2.166	7.3	9.466	169	10-Feb	15-Feb	135	140	34
CN7	1	4	4	25	2.079	7.4	9.479	133	17-Feb	20-Feb	142	145	37
CN7	1	4	5	25	2.117	7.7	9.817	151	18-Feb	24-Feb	143	149	47
CN7	2	1	1	23	2.268	9.6	11.868	139	7-Feb	13-Feb	132	138	26
CN7	2	1	2	23	2.359	5.6	7.959	136	2-Feb	6-Feb	127	131	19
CN7	2	1	3	23	3.314	7.6	10.914	164	3-Feb	8-Feb	128	133	25
CN7	2	1	4	22	2.493	7.6	10.093	137	5-Feb	11-Feb	130	136	18
CN7	2	1	5	24	2.991	7.3	10.291	172	4-Feb	11-Feb	129	136	30
CN7	2	2	1	23	1.679	4.5	6.179	115	1-Feb	5-Feb	126	130	23
CN7	2	2	3	22	2.513	6.9	9.413	148	3-Feb	8-Feb	128	133	21
CN7	2	2	4	23	1.756	5.4	7.156	115	4-Feb	7-Feb	129	132	29
CN7	2	2	5	23	2.467	6.4	8.867	150	5-Feb	11-Feb	130	136	19
CN7	2	3	2	24	1.506	7.9	9.406	142	11-Feb	16-Feb	136	141	15
CN7	2	3	3	23	1.244	6.4	7.644	138	11-Feb	16-Feb	136	141	15
CN7	2	3	4	25	1.465	8.5	9.965	178	17-Feb	27-Feb	142	152	36

CN7	2	3	5	25	1.376	6.9	8.276	185	18-Feb	22-Feb	143	147	16
CN7	2	4	1	24	1.252	5.7	6.952	120	14-Feb	19-Feb	139	144	19
CN7	2	4	2	24	1.507	6.9	8.407	128	15-Feb	19-Feb	140	144	23
CN7	2	4	3	23	1.965	6.7	8.665	156	12-Feb	15-Feb	137	140	13
CN7	2	4	4	24	2.596	10.4	12.996	180	8-Feb	16-Feb	133	141	29
CN7	2	4	5	25	2.129	5.5	7.629	126	19-Feb	24-Feb	144	149	44
CN8	1	1	1	23	2.251	9.3	11.551	137	5-Feb	11-Feb	130	136	13
CN8	1	1	2	24	2.357	6.8	9.157	139	5-Feb	14-Feb	130	139	18
CN8	1	1	3	23	2.124	7.6	9.724	155	10-Feb	14-Feb	135	139	20
CN8	1	1	4	24	3.749	9.9	13.649	141	13-Feb	18-Feb	138	143	20
CN8	1	1	5	24	2.531	10.3	12.831	152	9-Feb	18-Feb	134	143	20
CN8	1	2	2	23	2.828	7.3	10.128	245	8-Feb	13-Feb	133	138	23
CN8	1	2	3	25	2.556	7.7	10.256	162	11-Feb	16-Feb	136	141	10
CN8	1	2	4	24	2.414	7.5	9.914	105	12-Feb	16-Feb	137	141	14
CN8	1	2	5	24	1.531	5.5	7.031	146	11-Feb	15-Feb	136	140	14
CN8	1	3	1	25	1.516	6.7	8.216	139	14-Feb	20-Feb	139	145	15
CN8	1	3	2	27	2.657	7.3	9.957	111	17-Feb	2-Mar	142	155	7
CN8	1	3	3	25	1.415	7.1	8.515	160	18-Feb	25-Feb	143	150	11
CN8	1	3	5	25	2.594	7.1	9.694	147	19-Feb	24-Feb	144	149	14
CN8	1	4	1	25	2.855	7.4	10.255	147	19-Feb	24-Feb	144	149	10
CN8	1	4	3	25	3.091	8.9	11.991	151	19-Feb	23-Feb	144	148	16
CN8	1	4	4	25	2.701	9.8	12.501	153	22-Feb	2-Mar	147	155	28
CN8	1	4	5	25	2.198	8	10.198	141	21-Feb	26-Feb	146	151	17
CN8	2	1	1	24	3.037	7.3	10.337	143	12-Feb	19-Feb	137	144	17
CN8	2	1	2	25	2.456	6.5	8.956	106	9-Feb	15-Feb	134	140	23
CN8	2	1	3	24	3.333	7.5	10.833	129	9-Feb	19-Feb	134	144	21
CN8	2	1	4	25	1.813	7.7	9.513	127	11-Feb	20-Feb	136	145	30
CN8	2	1	5	24	3.457	11.3	14.757	169	7-Feb	11-Feb	132	136	25

CN8	2	2	1	24	2.284	5.3	7.584	129	9-Feb	15-Feb	134	140	13
CN8	2	2	2	24	2.162	5.4	7.562	105	8-Feb	17-Feb	133	142	9
CN8	2	2	3	24	2.886	8.5	11.386	115	6-Feb	8-Feb	131	133	17
CN8	2	2	4	24	1.713	6	7.713	131	11-Feb	16-Feb	136	141	10
CN8	2	2	5	25	1.828	6.9	8.728	161	12-Feb	21-Feb	137	146	15
CN8	2	3	3	25	1.938	7.1	9.038	167	20-Feb	27-Feb	145	152	19
CN8	2	3	4	25	1.873	6.5	8.373	136	20-Feb	24-Feb	145	149	18
CN8	2	3	5	25	2.522	9.3	11.822	168	19-Feb	25-Feb	144	150	15
CN8	2	4	1	25	1.979	8.5	10.479	171	17-Feb	23-Feb	142	148	12
CN8	2	4	3	24	2.441	7.7	10.141	162	17-Feb	22-Feb	142	147	17
CN8	2	4	5	24	1.519	9	10.519	140	19-Feb	26-Feb	144	151	22
J1	1	1	1	20	1.395	5.3	6.695	100	20-Jan	24-Jan	114	118	19
J1	1	1	2	19	2.534	6.9	9.434	105	22-Jan	23-Jan	116	117	24
J1	1	1	3	18	1.987	5	6.987	89	21-Jan	29-Jan	115	123	13
J1	1	1	4	20	1.612	4.5	6.112	100	21-Jan	25-Jan	115	119	19
J1	1	1	5	20	2.314	7.1	9.414	115	20-Jan	22-Jan	114	116	11
J1	1	2	1	19	1.845	4.4	6.245	78	5-Jan	8-Jan	99	102	33
J1	1	2	2	21	2.562	6.9	9.462	97	22-Jan	25-Jan	116	119	26
J1	1	2	3	19	1.473	4.6	6.073	113	10-Jan	13-Jan	104	107	28
J1	1	2	4	19	1.527	4.7	6.227	82.5	8-Jan	10-Jan	102	104	27
J1	1	2	5	21	1.617	4.9	6.517	68	26-Jan	28-Jan	120	122	14
J1	1	3	1	20	1.965	5.8	7.765	90	27-Jan	28-Jan	121	122	19
J1	1	3	2	22	2.357	7.2	9.557	134	5-Feb	7-Feb	130	132	22
J1	1	3	3	21	1.977	6.3	8.277	123	28-Jan	11-Feb	122	136	27
J1	1	3	4	21	2.638	6.7	9.338	101	22-Jan	23-Jan	116	117	21
J1	1	3	5	22	2.082	6.9	8.982	128	8-Feb	10-Feb	133	135	25
J1	1	4	1	22	2.427	5.5	7.927	96	5-Feb	9-Feb	130	134	28
J1	1	4	2	21	2.096	4.6	6.696	113	28-Jan	31-Jan	122	125	25

J1	1	4	3	22	3.119	8.3	11.419	134	5-Feb	8-Feb	130	133	33
J1	1	4	4	21	3.638	7.3	10.938	97	28-Jan	1-Feb	122	126	38
J1	2	1	1	16	1.529	5.1	6.629	80	7-Jan	10-Jan	101	104	32
J1	2	1	2	20	1.856	5.4	7.256	102	20-Jan	22-Jan	114	116	22
J1	2	1	3	20	2.082	6.5	8.582	133	18-Jan	21-Jan	112	115	19
J1	2	1	4	20	2.185	8.3	10.485	151	22-Jan	24-Jan	116	118	10
J1	2	1	5	20	2.354	8.3	10.654	129	13-Jan	17-Jan	107	111	29
J1	2	2	1	20	1.852	6	7.852	124	20-Jan	24-Jan	114	118	21
J1	2	2	2	20	2.343	6.5	8.843	104	20-Jan	22-Jan	114	116	41
J1	2	2	3	20	1.871	5.7	7.571	101	21-Jan	25-Jan	115	119	27
J1	2	2	4	19	1.35	3.8	5.15	72	9-Jan	11-Jan	103	105	24
J1	2	2	5	20	2.465	8.4	10.865	107	20-Jan	24-Jan	114	118	27
J1	2	3	1	23	2.234	6	8.234	119	8-Feb	10-Feb	133	135	25
J1	2	3	2	22	2.134	6.3	8.434	142	6-Feb	9-Feb	131	134	30
J1	2	3	3	23	2.196	6.5	8.696	118	6-Feb	9-Feb	131	134	36
J1	2	3	4	21	1.695	5.5	7.195	131	27-Jan	29-Jan	121	123	21
J1	2	3	5	22	2.003	7.2	9.203	140	5-Feb	10-Feb	130	135	20
J1	2	4	1	22	2.005	5.2	7.205	112	9-Feb	10-Feb	134	135	29
J1	2	4	2	22	2.813	7.5	10.313	129	6-Feb	10-Feb	131	135	43
J1	2	4	3	22	2.317	6.6	8.917	124	1-Feb	2-Feb	126	127	37
J1	2	4	4	21	2.177	6.1	8.277	104	22-Jan	1-Feb	116	126	29
J1	2	4	5	21	2.237	6.5	8.737	110	27-Jan	30-Jan	121	124	22

Appendix C

Summary statistics, analyses and brief discussion of growth chamber data not discussed in Chapter 4

Table C.1. Summary statistics for all plant characters measured in the growth chamber experiment (Chapter 4) for U.S. populations under the northern light treatment.

		Days to anthesis		Root biomass (g)		Aerial biomass (g)		Total biomass		# term. Infl.		Total height (cm)		
Pop	N Obs	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Latitude
SC	20	119.526	1.377	2.183	0.097	5.700	0.207	7.883	0.277	11.053	0.807	125.800	3.211	33° 48' 28" N
NC	20	123.850	2.292	1.644	0.069	5.870	0.378	7.514	0.413	11.579	0.796	137.350	2.629	35° 53' 24" N
VA1	20	103.800	1.224	1.651	0.090	5.560	0.256	7.211	0.328	10.850	0.802	112.950	2.237	38° 42' 22" N
MD	20	103.750	0.584	1.276	0.092	5.520	0.258	6.796	0.338	15.600	1.570	106.850	2.559	38° 47' 43" N
VA2	20	99.150	1.667	1.553	0.105	5.150	0.188	6.703	0.257	14.700	1.672	112.950	2.237	38° 57' 44" N
DE	19	94.947	2.545	0.812	0.055	3.837	0.278	4.648	0.297	16.895	2.334	92.711	3.602	39° 34' 22" N
WV	20	89.500	2.348	1.054	0.082	4.240	0.235	5.294	0.298	14.800	1.128	101.950	2.641	39° 39' 45" N
PA	20	85.600	0.887	1.171	0.100	4.215	0.237	5.386	0.317	13.000	1.021	107.025	2.988	40° 26' 05" N
NJ	20	79.000	0.718	1.130	0.089	3.735	0.196	4.865	0.252	16.400	0.659	104.825	2.306	40° 30' 44" N
CT	20	84.100	1.000	0.997	0.072	3.485	0.252	4.482	0.297	12.550	0.659	102.450	2.225	41° 18' 18" N

Table C.2. Summary statistics for all plant characters measured in the growth chamber experiment (Chapter 4) for U.S. populations under the southern light treatment.

		Days to anthesis		Root biomass (g)		Aerial biomass (g)		Total biomass		# term. Infl.		Total height (cm)		
Pop	N Obs	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Latitude
SC	20	70.105	0.657	1.690	0.106	4.680	0.251	6.370	0.343	10.900	0.900	114.850	3.190	33° 48' 28" N
NC	20	83.800	1.104	1.247	0.081	4.715	0.277	5.962	0.342	16.200	0.851	118.100	4.994	35° 53' 24" N
VA1	20	86.700	0.519	1.251	0.080	4.340	0.201	5.591	0.270	7.950	0.505	113.800	2.945	38° 42' 22" N
MD	20	105.700	0.941	1.170	0.065	4.915	0.245	6.085	0.305	16.200	0.622	103.225	1.811	38° 47' 43" N
VA2	20	71.150	0.539	1.153	0.110	3.710	0.272	4.863	0.356	13.211	0.836	104.075	3.452	38° 57' 44" N
DE	20	70.150	0.514	0.734	0.042	3.450	0.221	4.184	0.248	15.300	1.765	99.175	2.515	39° 34' 22" N
WV	20	102.700	0.442	0.957	0.068	3.560	0.153	4.517	0.201	14.050	1.040	99.950	2.719	39° 39' 45" N
PA	20	93.053	0.549	0.896	0.059	2.815	0.222	3.711	0.274	11.250	1.158	101.700	2.289	40° 26' 05" N
NJ	20	84.650	2.163	1.104	0.069	3.320	0.199	4.424	0.260	16.150	0.730	109.525	2.331	40° 30' 44" N
CT	19	75.650	1.769	0.917	0.054	2.963	0.200	3.880	0.242	10.895	0.985	104.000	2.203	41° 18' 18" N

Table C.3. Summary statistics for all plant characters measured in the growth chamber experiment (Chapter 4) for Asian populations under the northern light treatment.

		Days to anthesis		Root biomass (g)		Aerial biomass (g)		Total biomass		# term. Infl.		Total height (cm)		
Pop	N Obs	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Latitude
CN3	10	149.800	0.800	2.198	0.194	6.670	0.818	8.868	0.814	23.800	3.252	142.000	3.759	23 45' 37" N
CN1	17	131.588	1.407	1.781	0.088	7.465	0.408	9.246	0.480	19.941	1.739	145.294	3.355	23 55' 48" N
CN5	16	151.313	0.740	1.967	0.124	8.144	0.487	10.111	0.598	18.813	1.275	150.938	4.284	24 03' 24" N
CN8	14	149.929	0.745	2.236	0.144	7.886	0.279	10.121	0.356	15.786	1.403	149.500	4.285	24 03' 32" N
CN4	16	148.063	1.138	2.152	0.118	7.538	0.267	9.689	0.370	15.250	0.938	139.938	3.595	24 15' 48" N
CN7	16	144.188	0.905	1.772	0.117	7.038	0.302	8.810	0.367	26.750	2.939	149.875	4.985	24 18' 10" N
J1	19	129.895	1.331	2.322	0.105	6.421	0.204	8.743	0.282	27.895	1.571	118.158	3.563	34.51408 N

Table C.4. Summary statistics for all plant characters measured in the growth chamber experiment (Chapter 4) for Asian populations under the southern light treatment.

		Days to anthesis		Root biomass (g)		Aerial biomass (g)		Total biomass		# term. Infl.		Total height (cm)		
Pop	N Obs	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	Latitude
CN3	18	134.722	0.582	2.596	0.165	7.650	0.435	10.246	0.579	20.222	1.683	134.333	3.530	23 45' 37" N
CN1	17	121.706	1.080	1.746	0.135	6.300	0.380	8.046	0.499	19.059	1.142	129.941	4.030	23 55' 48" N
CN5	17	141.000	1.231	2.104	0.117	7.906	0.417	10.010	0.507	23.824	2.223	153.294	3.770	24 03' 24" N
CN8	19	134.368	0.553	2.490	0.139	7.595	0.381	10.085	0.479	17.474	1.280	141.947	7.228	24 03' 32" N
CN4	19	134.842	0.694	2.652	0.099	8.137	0.337	10.789	0.416	20.421	1.962	139.842	3.563	24 15' 48" N
CN7	18	127.444	0.776	2.109	0.135	6.217	0.381	8.326	0.486	21.000	1.694	137.056	4.733	24 18' 10" N
J1	20	111.250	1.369	1.938	0.089	5.915	0.311	7.853	0.389	23.300	1.755	102.525	4.756	34.51408 N

Table C.5. ANOVA results for the fixed effects of population origin, light treatment, and their interactions, and the random effects of experimental chamber, block, and their interactions on U.S. populations of *M. vimineum* final height and number of terminal inflorescences from the growth chamber experiment described in Chapter 4. 'Est' is the covariance parameter estimate and 'SE' in the standard error of the covariance parameter estimate. 'n/a' specifies that Wald Z values could not be calculated due to negative covariance estimates, which indicates that the random effect was not significant.

Source of variation	Num d.f.	Den d.f	Final height (cm)		# Term. Infl.	
			F	*P*	*F*	*P*
Fixed effects						
Pop	9	375	21.53	**<0.0001**	9.06	**<0.0001**
Treatment	9	375	9.86	**0.0018**	1.25	0.2641
PxT	1	375	3.61	**0.0002**	1.72	0.0832
Covariance parameter			Est	SE	Est	SE
Random effects						
Chamber			n/a	n/a	n/a	n/a
Block			n/a	n/a	n/a	n/a
C x B			n/a	n/a	**0.5743**	0.1414

Note: bold indicates significant differences (α=0.05).

Table C.6. ANOVA results for the fixed effects of population origin, light treatment, and their interactions, and the random effects of experimental chamber, block, and their interactions on Asian populations of *M. vimineum* anthesis, total biomass, aerial biomass, root biomass, final height, and number of terminal inflorescences from the growth chamber experiment described in Chapter 4. 'Est' is the covariance parameter estimate and 'SE' in the standard error of the covariance parameter estimate. 'n/a' specifies that Wald Z values could not be calculated due to negative covariance estimates, which indicates that the random effect was not significant.

Source of variation	Num d.f.	Den d.f	Days to anthesis		Total biomass (g)		Aerial biomass (g)		Root biomass (g)		Final height (cm)		# Term. Infl.	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Fixed effects														
Pop	6	219	168.03	**<.0001**	6.72	**<.0001**	7.37	**<.0001**	7.66	**<.0001**	19.41	**<.0001**	6.73	**<.0001**
Treatment	6	219	254.69	**<.0001**	2.77	0.0977	5.49	**0.02**	6.43	**0.0119**	8.56	**0.0038**	1.12	0.2906
PxT	1	219	5.41	**<.0001**	1.98	0.0698	1.81	0.098	23.09	**0.0064**	1.31	0.2543	3.1	**0.0062**
Covariance parameter			Est	SE	Est	SE	Est	SE	Est	SE	Est	SE	Est	SE
Random effects														
Chamber			n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.8182	0.7404
Block			n/a	n/a	0.1527	0.0786	**0.1521**	0.06534	n/a	n/a	0.64	0.6148	n/a	n/a
C x B			n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.1042	0.0926

Note: bold indicates significant differences (α=0.05).

Table C.7. ANOVA results for the fixed effects of light treatment, seed origin (U.S. or Asia) and their interactions, and the random effects of experimental chamber, block, and their interactions on all samples (U.S. and Asian) of *M. vimineum* anthesis, total biomass, aerial biomass, root biomass, final height, and number of terminal inflorescences from the growth chamber experiment described in Chapter 4. 'Est' is the covariance parameter estimate and 'SE' in the standard error of the covariance parameter estimate. 'n/a' specifies that Wald Z values could not be calculated due to negative covariance estimates, which indicates that the random effect was not significant.

Source of variation	Num d.f.	Den d.f	Days to anthesis		Total biomass (g)		Aerial biomass (g)		Root biomass (g)		Final height (cm)		# Term. Infl.	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Fixed effects														
Treatment	1	626	27.59	**<.0001**	631.93	**0.0052**	12.89	**0.0004**	0.33	0.568	10.17	**0.0015**	1.85	0.1738
Region	1	626	1852.74	**<.0001**	7.88	**<.0001**	582.22	**<.0001**	463.22	**<.0001**	354	**<.0001**	2.95	0.0862
R x T	1	626	5.33	**0.0212**	12.73	**0.0004**	7.77	**0.0055**	23.88	**<.0001**	1.78	0.1822	0.35	0.557
Covariance parameter			Est	SE	Est	SE	Est	SE	Est	SE	Est	SE	Est	SE
Random effects														
Chamber			0.7826	0.7556	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Block			n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
C x B			n/a	n/a	0.018	0.0146	**0.02997**	0.01366	n/a	n/a	n/a	n/a	n/a	n/a

Brief Discussion of Some Analyses Presented in Appendix C

In Chapter 4, I presented and detailed the significance of the clinal variation in growth patterns and phenology observed in *M. vimineum* populations in its invasive range (North America). In Chapter 4, I relied on only a subset of the total characters that I measured in the growth chamber experiment to make the case for adaptive evolution. Additionally, I only discussed the data from North American samples, even though I also assayed many samples from the Asian range. In Appendix C, I reported summary statistics for all variables measured for all population. It is important to note that I did start with ten populations from Asia (9 from Yunnan region and 1 from Japan). Three of the populations from Yunnan (CN2, CN6, and CN9) were either heavily or completely contaminated with seeds from other species of *Microstegium* or *Arthraxon*, which only became apparent after plants began to mature. Consequently those populations were removed from the experiment.

Most of the data from the North American populations was reported in Chapter 4. The only two variables not presented or discussed were total height and the number of terminal inflorescences on each plant. The total height variable (see Tables C.1 and C.2) essentially mirrored the total biomass variable. Total height, like biomass, decreased as latitude increased, similarly to the tradeoff between biomass and flowering time discussed in Chapter 4. ANOVA analyses (Table C.5) predictably showed that seed origin was the major source of plant height variation with light treatment playing a small role as well (presumably since each treatment received different total photonic energy

amounts). The variable for the number of terminal inflorescences, however, did not tell the same story as the biomass variables. The variation in the number of terminal inflorescences was significantly determined by seed source, but not light treatment (Table C.5), indicating genetic determination of the number of terminal inflorescences. This pattern could also be the result of a maternal effect, but that seems unlikely considering this is essentially a morphological trait that would not be dependent on the mother plant's health or vigor (as many maternal effects, such as seed size, tend to be). Moreover, the variation in the number of terminal inflorescences did not seem to be dependent on latitude of the seed origin. In fact, regression analyses relating number of terminal inflorescences and latitude of seed origin, for the northern and southern light treatments separately, revealed no significant relationship. This indicates that although the number of terminal inflorescences is genetically determined, it is not related to latitude or phenology. As a variable genetic trait, it may be responding to some local selection pressure. It would be interesting to determine if the number of terminal inflorescences is related to overall fecundity. It would also be interesting to determine if this character results in increased outbreeding since there would seem to be more chasmogamous flower production in plants that have more terminal inflorescences. Unraveling the determinants of this trait could have important implications for adaptive evolutionary processes in *M. vimineum*.

Tables C.3 and C.4 present the summary statistics for all variables measured in the growth chamber experiment for all Asian populations. It is important to note that the Asian samples did not represent a well distributed

latitudinal cline. Because I was limited to seed lots that were sent to me from collaborators, I only obtained seed from Yunnan region in China and central Japan. Therefore, robust tests of latitudinal clines were not possible. Nevertheless, it was apparent that biomass measures and plant height were generally much higher in Yunnan than Japan and that Japanese plants flowered sooner than plants from Yunnan. Although these general relationships resembled those observed in North American samples, there was considerable variability of the biomass measures within Yunnan such that Japanese samples actually had a higher average root biomass than some Yunnan samples. For the other biomass measures, Japanese samples averaged the lowest biomass but not by much, especially when considering the over ten degrees of latitude difference from Japan to Yunnan. This dampened latitudinal response could reflect climatic differences of Japan as an island vs. inland China or could reflect the fact that these are lower temperate to sub-tropical latitudes which are all further south than all but one of the North American populations sampled. Once again, the number of terminal inflorescences showed great variability that did not seem related to latitude (Table C.3 and C.4) nor was it dependent on light treatment (Table C.6), highlighting the need for further study of this trait as it seems to be highly variable and genetically controlled. The selective pressures which mold its variation are unclear yet may be related to the relative abundance of cleistogamous and chasmogamous seeds on each plant.

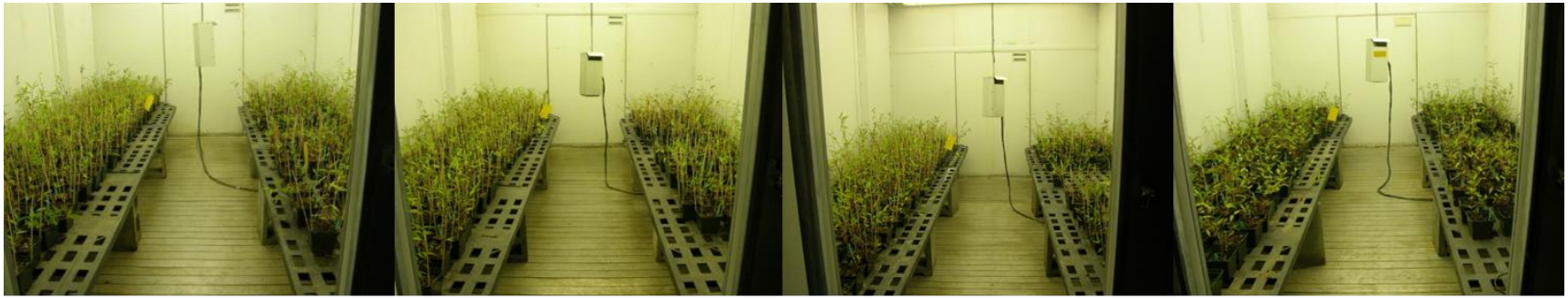
I also conducted ANOVAs for all variables including all samples from both North America and Asia to determine if there were differences resulting from

invasive vs. native plants (Table C.7). Days to anthesis, biomass variables and final height all showed significant variance resulting from both the continent of seed origin and the light treatment. Once again, the fact that the light treatment was significant is not surprising due to the expected consequences of differential light exposure (and amount of heat) on these characters. It is somewhat surprising that plants from the different regions behaved differently. However, once we remember that these seeds came from very different latitudinal environments (approx. 34-41° for North America) and (approx. 24-35° for Asia), these different behaviors may be explained. As Colautti et al. (2009) found, including latitude into statistical models of plant life history and physiological traits for 28 invasive species reduced or nullified differences between plants from native vs. invasive sources. Terminal inflorescence number (Table C.7) was not significantly affected by either region or treatment. Since individual population origin, in both the native and introduced ranges, seems to be the only determinant of this seemingly genetically determined variable based on the differing conditions in this experiment, the selection pressure creating the differentiation seen here remains an intriguing open question.

Appendix D

Photos from the growth chamber experiment including some interesting phenotypes observed

Figure D.1 Images of plants in the growth chamber experiment. Chambers 1-4 are shown from left to right. The top line of images represents plants at approximately 2 months of growth and the bottom lines represent plants at approximately 4 months. Plants in the center isle in the bottom row have senesced and were removed for measurements that day.



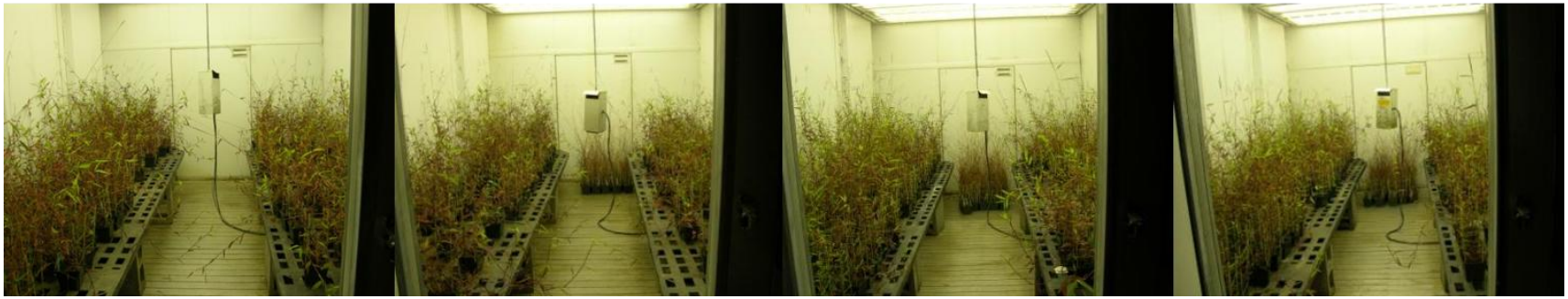


Figure D.2. An example of *M. vimineum* at first flowering. Note how the first pair(s) of anthers had exerted on this day.

Figure D.3. Two distinct anther colors were noted for *M. vimineum* flowers during the growth chamber experiment. All of the North America anthers displayed the brownish-red anther phenotype. All Japanese individuals had yellow anthers. Most Chinese samples had yellow anthers though some individuals from each of the Chinese populations displayed brownish or intermediate color anthers. This serves as some indication that Japan may not be the origin of invasion for North American *M. vimineum*. I could find no references in the literature as to the adaptive significance of anther color in wind pollinated grasses.

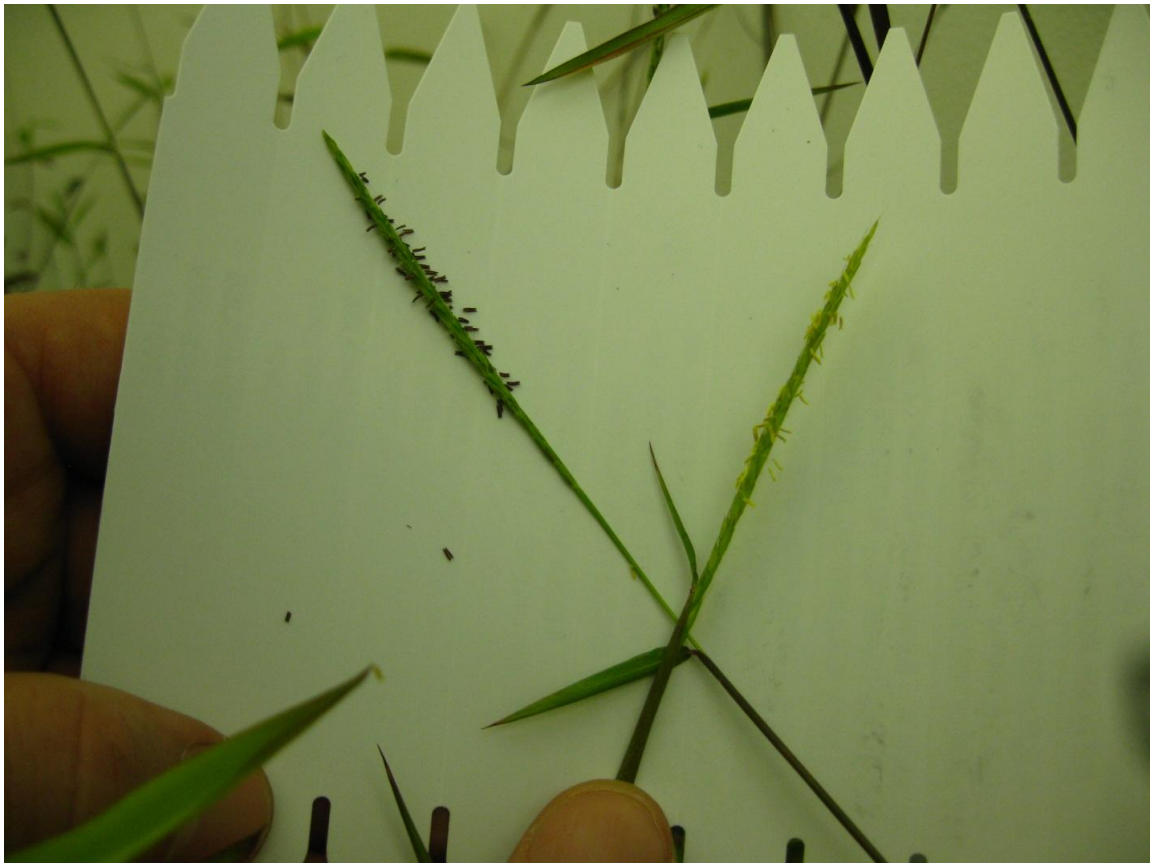


Figure D.4. Of all plants grown in the growth chamber experiment (approx. 740), this individual from the CN7 population in Yunnan, China was the only mutant phenotype observed. It originally presented with highly reduced growth and severely anthocyanic leaves and stems. Eventually, it grew out of the anthocyanic phase and demonstrated the decreased internode length and chlorotic streaking evident in this photo. The plant is approximately 6.5 months old in this photo. The plant never grew to more than 5 cm and seemed insensitive to light signals for flowering. After the experiment ended and all other plants had senesced, this plant was still alive and growing. I transported it to a windowsill where it did eventually produce cleistogamous seeds (but no chasmogamous seeds) and senesce, after over 8 months of growth.

Figure D.5. One of the student assistants, Chris Jurgensen, standing with the tallest plant from the North American rage. This plant was from North Carolina and grew to 171 cm under the southern light treatment. The tallest overall plant in the experiment was from Yunnan, China (CN8) and grew to 245 cm.

Figure D.6. A terminal spike of *M. vimineum* emerging already fertilized. In other words, this plant was fully cleistogamous. Even its terminal inflorescence was cleistogamous (i.e., shed pollen and selfed before the flowers emerged from the leaf sheath). I observed less than 5 plants with this feature, all from China.

Figure D.7. An example of a *M. vimineum* plant with anthocyanic surface roots. Several plants from several different populations in Asia and North America displayed this phenotype. The fact that differential root phenotypes (i.e., anthocyanic and whitish/yellow) emerged under common garden conditions may indicate genetic control of such traits with potential adaptive significance.

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Curriculum Vitae

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PEER REVIEWED PUBLICATIONS

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