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Invasive *Wisteria* in the Southeastern United States: genetic diversity, hybridization and the role of urban centers

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Abstract The increasing numbers and negative impacts of invasive species have prompted research on the relationship between human activities and the success of invasive horticultural plants. In this study, we use population genetic relationships to model the escape of a common garden vine, exotic *Wisteria,* into natural habitats. Urban and naturalized *Wisteria* populations in Charleston, South Carolina and Tallahassee, Florida were investigated using a combination of chloroplast, mitochondrial and nuclear DNA markers. Fifty-nine of 72 (81.9%) *Wisteria* collections were hybrids of *Wisteria sinensis* and *W. floribunda*. Chi-square analysis of the distribution of shared *W. floribunda* haplotypes among naturalized and urban populations supports the relationship of time with invasion success. Naturalized populations were more similar to those in historic neighborhoods. The most common haplotype, F1, was encountered 22 times but its distribution was not significantly different between urban and naturalized populations. In contrast, a significantly higher proportion of haplotype F2 found in naturalized populations suggests that selection may also be acting within these populations. Finally, due to extensive human dispersal, there is no relationship between genetic distance and geographical distance among the populations sampled. We conclude that *Wisteria's* long history of horticulture, rampant hybridization, and human-aided dispersal are all implicated in the successful ability of these plants to invade natural habitats throughout the USA.

Keywords Haplotype analysis · Horticulture · Invasive species · Sequence characterized amplified region (SCAR) · Urbanization

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Introduction

Many exotic plant species are purposefully introduced by humans. In the eastern USA, 61–68% of plants that were deliberately introduced before 1900 have become naturalized (Mack and Erneberg 2002). Reichard and Campbell (1996) found that over 50% of all US invasive plants were introduced for horticultural or ornamental purposes. The economic importance of non-native horticultural species is clear; nearly 60,000 species and varieties of plants are offered by North American nurseries alone (Ewel et al. 1999). A 1998 estimate accounted for 10.6 billion dollars in US horticultural sales (USDA-NASS 1998).

Horticultural introductions are most diverse in urban environments where human settlement is most dense (Kowarik 1990). Cities are often economic focal points and provide entry points for many invasive species. Many invasive species are most prolific in urban and suburban environments where human disturbances have created abundant opportunities for invasion (McNeely 2005). Human settlement patterns, along with the creation of transportation corridors, aid in the spread of invasive species (Marambe et al. 2001).

Although many non-native plants species are introduced, only a fraction of these species eventually become invasive (Williamson and Fitter 1996). Introduced species are subject to new physical and biotic communities with altered selection pressures (Cox 2005). These pressures select upon the genetic diversity of an introduced organism and often cause rapid genetic changes (Thompson 1998). It is only those species that are able to respond positively to these new biotic and abiotic regimes that become invasive. The ability to adapt is directly linked to an organism's genetic diversity. High genetic diversity arrives with an introduced plant species if the founding population is large enough or there are multiple introduction events (Rowe et al. 1997). In addition, genetic diversity can be gained through hybridization. Many weedy species gain genetic variability by hybridizing with closely related species (Ellstrand et al. 1999). Hybridization can occur between species or ecotypic races that are geographically isolated in their native ranges (Ellstrand and Schierenbeck 2000).

Urban environments are an important link in the creation of invasive species. Urban areas hold numerous species, many of which were once geographically isolated, in a small localized area. This close proximity increases the potential for augmentation of genetic diversity through inter and intra species hybridization events. The new hybrid taxon can use this increased genetic diversity to respond to the selection pressures of its new home. In addition, cities represent "genetic experiments" that are repeated both in time and among urban areas spread throughout the world. Each experiment differs in that the introduced species is subject to the unique selective environment of its new urban home.

In order to reduce the negative economic and ecological impacts of invasive plant species for the future, an understanding how human activities and urban environments promote the adaptation, dispersal and abundance of invasive species must be gained.

Invasive Wisteria: A model system

Both Chinese (*W. sinensis* (Sims) DC.) and Japanese (*W. floribunda* (Willd.) DC.) *Wisteria* were introduced to the USA between 1830 and 1860 as ornamental plants and a number of cultivars are still important in the horticulture trade (Remaly 1999). The ease of propagation, showy springtime floral display and vigorous growth led to the widespread introduction of exotic *Wisteria* into urban and residential areas throughout the USA. To

date, exotic *Wisteria* species have been listed as invasive pest plant species in five southeastern states (USDA-NRCS 2004). In South Carolina, invasive *Wisteria* is tied as the fifth most commonly encountered exotic on forested lands (Oswalt 2005).

Preliminary research conducted in our lab has shown that naturalized plants throughout the southeastern USA are cryptic hybrids between *W. floribunda* and *W. sinensis* (Trusty et al. 2007). This new hybrid species has managed the remarkable journey out of the garden and into natural and managed forests, riparian areas, roadsides and parks throughout the southeast. Invasive *Wisteria* is a model system for understanding the evolutionary and ecological processes involved in the invasion of a horticultural plant species. First, invasive *Wisteria* is a novel hybrid species, easily distinguished with genetic markers from its parent species (Trusty et al. 2007). Second, the processes of hybridization and selection of ecologically fit genotypes have been replicated throughout the southeast. Finally, *Wisteria* have very large seeds with a low dispersal distance (Miller 2003). In order for *Wisteria* to escape from urban gardens into natural and managed forests, they had to succeed in a range of urban to rural land use types. Using introduced *Wisteria* populations in the southeastern USA, we have identified the population genetic signatures predicted by passive and active mechanisms of invasion from two urban centers.

Invasiveness and the role of urban centers

The study of plant invasions has identified both passive and active mechanisms that can result in the spread of an introduced species (Powell and Zimmerman 2004). Invasions may be termed as passive if the invasion is predicted by the length of time since first introduction or if it is due to the number of plants introduced. Under the passive invasion hypothesis, species that have been present for long periods of time and those that are numerically common are predicted to invade. In contrast, an active invasion is due to selection events within the introduced range. In this scenario, there is a phenotype within the population of an introduced species that is best suited for the new habitat or climate, is more fecund and therefore spreads easily. This phenotype may be naturally occurring or potentially a novel hybrid species (Mooney and Cleland 2001).

Under a scenario of passive invasion by exotic *Wisteria* either: (1) the time since introduction or (2) the initial population size is related to invasion. It is hypothesized that the longer the time since introduction or the larger the initial population size, the more likely the chance of invasion. If naturalized *Wisteria* populations are related to plants found in the oldest residential areas (representing the oldest individuals), it would indicate that the invasive *Wisteria* genotypes are simply those that have been around the longest. In contrast, if naturalized *Wisteria* populations belong to the most common *Wisteria* genotype, this would suggest that plant density is key in the escape of this species.

If *Wisteria* has naturalized through an active mechanism, we would expect to find that the invasive populations are related to an invasive genotype. This invasive genotype may be a small subset of the available genetic diversity or may be a novel hybrid genotype. Under the active invasion scenario, we hypothesize that there would be high genetic diversity of planted urban *Wisteria* and lower diversity in naturalized populations. It is possible that naturalized populations in different cities would share invasive genotypes.

In this study we investigated the identity and genetic diversity of planted and naturalized *Wisteria* in and around two southeastern US cities, Charleston, South Carolina and Tallahassee, Florida. Using nuclear DNA haplotype and GIS data, we have determined the genetic and spatial relationships of naturalized plants in order to elucidate active and/or passive mechanisms of invasion from urban horticultural populations.

Methods

Collections of *Wisteria* populations were made in and around two urban centers, Tallahassee, Florida, and Charleston, South Carolina. Collections were made in residential areas of two ages: historic neighborhoods before the turn of the twentieth century and recent (1950–1970s) urban expansion. The two neighborhood ages were surrogates for plant age as *Wisteria* do not have growth rings or other easily interpretable age diagnostics. In addition, plant collections were made in nearby naturalized locations between 1 and 40 km of the urban center. Locations of all collections were mapped using GPS coordinates (Figs. 1 and 2). A reference collection of *Wisteria* species from their native ranges was made from wild-collected plants housed in botanical garden living collections. Plant material of named horticultural varieties of *Wisteria* were provided from the private collection of Scott Lathrop (Santa Ana, CA) and used as additional reference species. Details of the plant material, Genbank accession number, and voucher information of the taxa sampled in this study are listed in Appendix 1. DNA was extracted from fresh or silica dried leaf samples using the CTAB protocol (Doyle and Doyle 1987).

Nuclear data

In order to discover variable DNA regions that were consistent between wild-collected *W. floribunda* and *W. sinensis* individuals, sequence characterized amplified regions (SCARs)

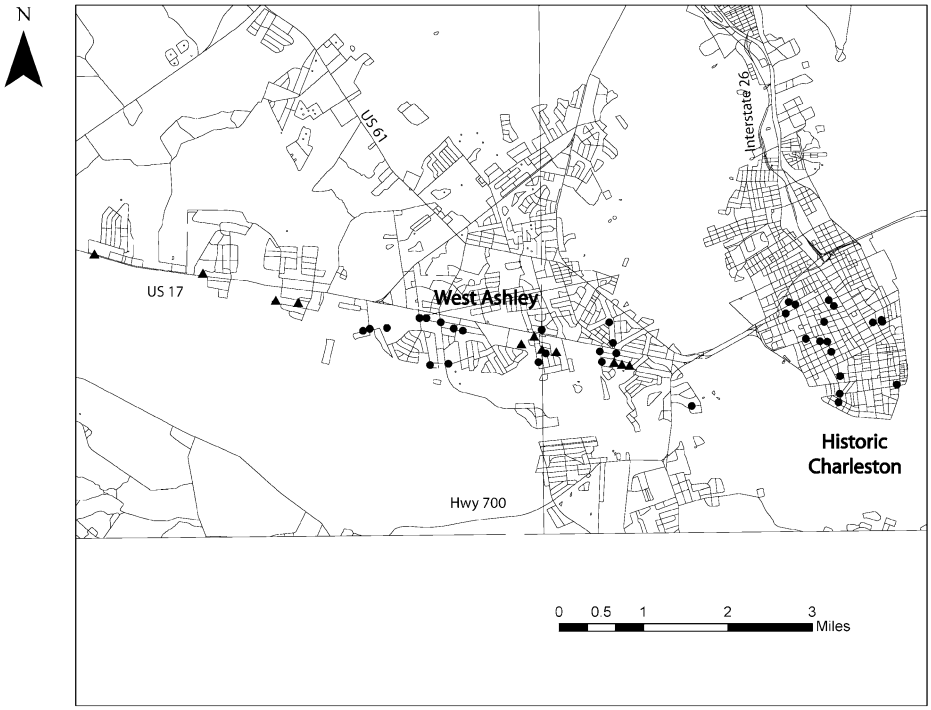

Fig. 1 Map of Charleston collection locations. Historic Charleston neighborhood dates prior to 1900s; West Ashley neighborhood represents 1950–1970 urban expansion. *Circles* represent urban locations; *triangles* are naturalized plants

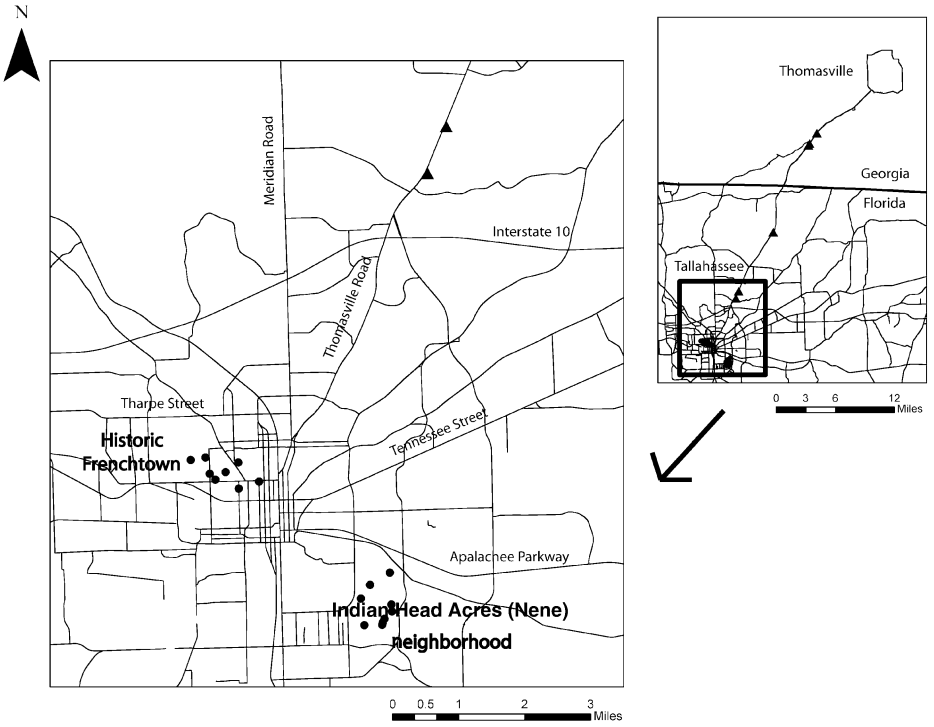

Fig. 2 Map of Tallahassee collection locations. Frenchtown neighborhood dates prior to 1900s; Nene neighborhood represents 1950–1970 urban expansion. *Circles* represent urban locations; *triangles* are naturalized plants

were developed from two ISSR primers. Primers (CA)6-RY and (CT)8-RA were used to amplify *W. sinensis* (Kew), and *W. floribunda* (Kew) using the following PCR reaction conditions: 0.4 UM primer, 1X Taq polymerase buffer, 0.2 μM dNTPs, 0. 25 U Taq polymerase (Eppendorf) and 1 μl of DNA in a 25 μl volume. Products were amplified using an MJ Research DNA Engine with the thermocycler program as in Wolfe et al. (1998). Five microliters of the reaction volume were run out on a 1% agarose gel containing 4 μl ethidium bromide in 1X sodium borate buffer and visualized on a UV transilluminator. One microliter of the ISSR PCR product was cloned for each species using the TOPO-TA cloning kit with plasmid vector pCR2 according to the manufacturer's protocol (Invitrogen). Colonies were screened for inserts using the M13F and M13R cloning primers and the thermocycler program described in the cloning kit. PCR products of clones larger than 400 bp were cleaned using Microcon PCR filter units (Millipore). Clones were sequenced in two directions with the cloning primers using the dideoxy chain termination method with ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems Inc.). Sequences were obtained by the Auburn University Genomics and Sequencing Lab on an ABI 3100 automated sequencer following manufacturer's instructions.

Cloned sequences of similar sizes (within 20 bp) from each of the three species were aligned manually. A single variable region was chosen from each original ISSR primer and specific primers w898-824F (CATGTTGCATTCAATCTTGG), w898-824R (GCCTCCA TACAAGTTAGTTG), w843-997F (GAATCAACGCTGAACGTT), and w843-997AluR

(GGTTCAATTTATTGATGTG) were designed. These primers were used to amplify all the samples used in this study with the following PCR reaction conditions: 0.4 μM forward primer, 0.4 μM reverse primer, 1X Taq polymerase buffer, 0.2 μM dNTPs, 0. 25 U Taq polymerase and 1 μl of DNA in a 25 μl volume. Thermocycler conditions were 94°C for 1 min; 35 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 1 min; followed by 72°C for 10 m.

Nuclear Region 824

Amplified products of nuclear region 824 were cloned and sequenced as above. Two to four clones were sequenced in two directions for each sample. Cloned sequences of region 824 were a total of 639 bp in length and were aligned manually. There was a single phylogenetically informative (6 bp) insertion in *W. brachybotrys* Siebold & Zucc. haplotypes of this region. *Wisteria frutescens* (L.) Poir. could not be amplified for region 824. Haplotypes were classified as identical only if they matched at every sequence character. Each sample had either one (homozygous) or two (heterozygous) haplotypes corresponding to a diploid chromosome number within *Wisteria*. The sequence data were imported into the program TCS 1.13 (Clement et al. 2000) and a haplotype network was generated using the 95% statistical parsimony limit.

Nuclear Region 997

Amplified products of nuclear region 997 were approximately 400 bp and contained a single HpyCH4 IV cut site at bp 84 in *W. floribunda* haplotypes. For all individuals, 5 μl of amplified product was cut for 1 h at 37°C in the following conditions: 0.5 μl HpyCH4 IV enzyme (New England Biolabs), 2 μl of 10X NEB buffer 1 and 12.5 μl of water. Twenty microliters of the reaction volume were run out on a 1.5% agarose gel containing 4 μL ethidium bromide in 1X sodium borate buffer and visualized on a UV transilluminator. Haplotypes were scored as *sinensis* (single, uncut band), *floribunda* (two, smaller cut bands) or hybrid (all three bands).

Chloroplast data

The chloroplast *trnL* gene and *trnL-trnF* intergenic spacer (*trnL/F*) of wild collected *W. floribunda* and *W. sinensis* were amplified using the 'C' and 'F' primers according to the protocol described in Taberlet et al. (1991). Products were sequenced using the amplification primers as described above. Primers WistrnLF (AGTTGACGACATTTCCTTAC) and WistrnLR (GGAGTGAATGGTTTGATCAATG) were designed to amplify a 250 bp region that contains a 30 bp deletion in *W. sinensis* taxa. Products for all samples were amplified using the Taberlet et al. (1991) protocol and visualized on a 1.5% agarose gel. Bands were scored by size as *floribunda* (F) or *sinensis* (S).

Mitochondrial data

Three hundred and eighty-five base-pairs of the mitochondrial NAD4 gene that contains a Sal I restriction enzyme cut site in *W. floribunda* were amplified using primers NAD4RSF1 (CTACTAGACTACTAGAGGT) and NAD4RSR1 (GTTTGGCAACAAGCAAACG) according to the protocol described in the nuclear data section above. Five microliters of PCR product were cut with 0.5 μl Sal I enzyme (New England Biolabs), 2 μl of NEB

buffer 3 and 12.5 μl of distilled water for 1 h at 37°C and visualized on a 1.5% agarose gel containing 4 μl ethidium bromide in 1X sodium borate buffer on a UV transilluminator. Bands were scored by size as cut or uncut; indicating *floribunda* (F) or *sinensis* (S) respectively.

Network and statistical analysis

Nuclear region 824 sequence data were imported into the program TCS 1.13 (Clement et al. 2000) and haplotype networks were generated for each city using the 95% statistical parsimony limit. Proportions of hybrid individuals between cities and among neighborhoods were compared with Fisher's exact test using the PROC FREQ statement in the program SAS version 9.1 (Cary, NC). Pairwise genetic distance (p) among *floribunda* haplotypes was calculated in Paup 4.0b* (Swofford 2002). Pairwise geographic distance between individuals was calculated in ArcView 9.1 (ESRI) using the Hawthe's Animal Tool extension. Regression analysis of genetic distance (p) with geographic distance was performed in program SAS using the PROC REG statement.

Results

Hybrid Wisteria prevalence

The majority of both naturalized and urban *Wisteria* sampled in our study were hybrids. Fifty-nine of the total of 72 (81.9%) *Wisteria* sample collections were hybrids of *Wisteria sinensis* and *W. floribunda* (Table 1). One collection was identified as *W. floribunda*, ten as *W. sinensis* and two as the native species, *W. frutescens*. Two collections were hybrids between *W. brachybotrys* and *W. floribunda* and *W. sinensis* respectively (data not shown). No hybridization between native and exotic *Wisteria* was found.

Compilation of the chloroplast *trnL-F*, mitochondrial *NAD4* and nuclear regions 824 and 997 shows that most of the hybrid collections are complex (F2 or later) hybrids (Appendix 1). Fifty-five of 59 (93%) *W. floribunda–W. sinensis* hybrid samples were fixed for at least one nuclear haplotype for the opposite parent as indicated by the plastid data. Fifty-two out of 59 (88%) hybrid collections had mitochondrial haplotypes of *W. sinensis*, showing a preference for directional hybridization with Chinese *Wisteria* as the maternal parent. In contrast, only 32 of 59 (54%) had chloroplast haplotypes of *W. sinensis*. Paternal origin of *Wisteria* chloroplasts has been previously suggested (Hu et al. 2005).

Genetic diversity

Sixty-six distinct haplotypes were identified and deposited into GenBank (Appendix 1). Sixteen haplotypes of *W. sinensis,* 50 haplotypes of *W. floribunda* and a single *W.*

Table 1 Identity of *Wisteria* individuals sampled. Row percentages shown

	W. floribunda	W. sinensis	W. frutescens	Hybrid	Total
Charleston	1	3	1	43	48
Tallahassee	0	7	1	16	24
Total	1 (1%)	10 (14%)	2 (3%)	59 (82%)	72

brachybotrys haplotype were found. The high degreee of DNA divergence between *W. sinensis* and *W. floribunda* haplotypes resulted in the creation of two separate networks, the upper network represents haplotypes of *W. sinensis* and the bottom of *W. floribunda* (Figs. 3 and 4). All but three of the *W. sinensis* haplotypes were a single base-pair different from the most common haplotype (the inferred ancestral haplotype). Two *sinensis* haplotypes were found in both cities: S1 and S2. The China-collected *W. sinensis* individual was homozygous for this inferred ancestral haplotype. In contrast, the *W. floribunda* haplotypes had a much higher diversity which ranged from one (0.15%) to 17 (1.72%) base-pair differences from the most common (inferred ancestral) haplotype. Neither of the two Japan-collected *W. floribunda* samples carried the most common haplotype. The *floribunda* haplotype tree for Charleston contained 43 different haplotypes (Fig. 3); the most common haplotype was encountered 12 times. The two next most common haplotypes were found at a frequency of 8 and 5 respectively. The *floribunda* haplotype tree for Tallahassee contained 15 different haplotypes (Fig. 4). The most common haplotype was found nine times and the next most common haplotype was found three times. Three *floribunda* haplotypes were found in both cities; F1, F2, and F3.

Relationship of naturalized Wisteria to urban centers

In Charleston, 43 of 47 (91.5%) exotic *Wisteria* collections are hybrids while only 16 of 23 (69.6%) are hybrid in Tallahassee (Table 1). Fisher's exact test indicates that the frequency

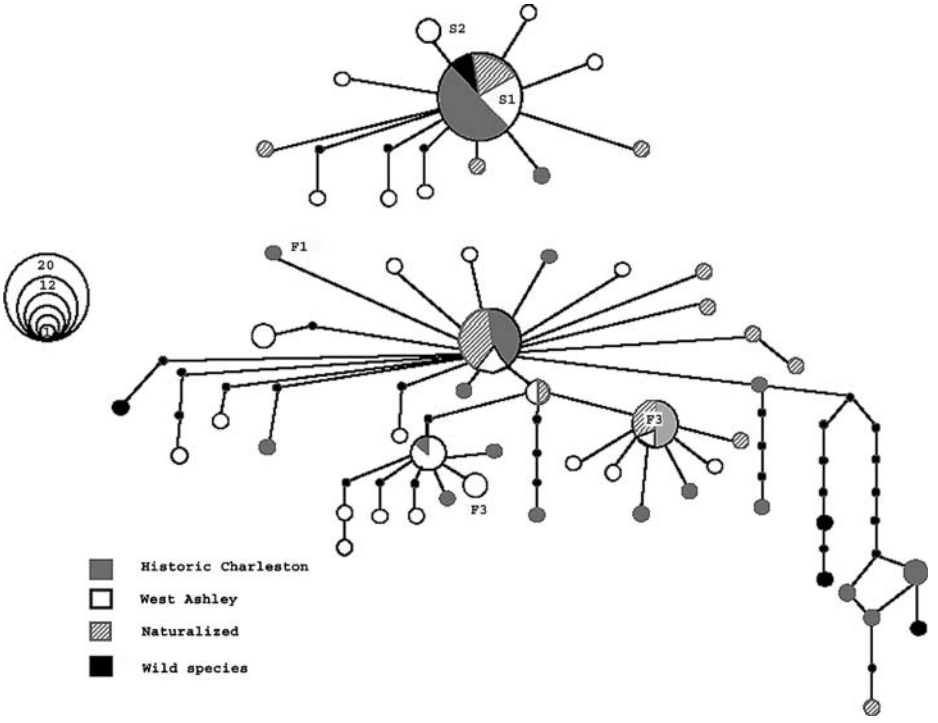

Fig. 3 Haplotype networks for nuclear region 824 of Charleston *Wisteria* samples. Top network contains all the *W. sinensis* haplotypes; bottom network are *W. floribunda* haplotypes. Each *branch length* implies a single mutational difference and *black dots* represent unsampled haplotypes. The *size of the circle* is proportional to the number of haplotypes recovered

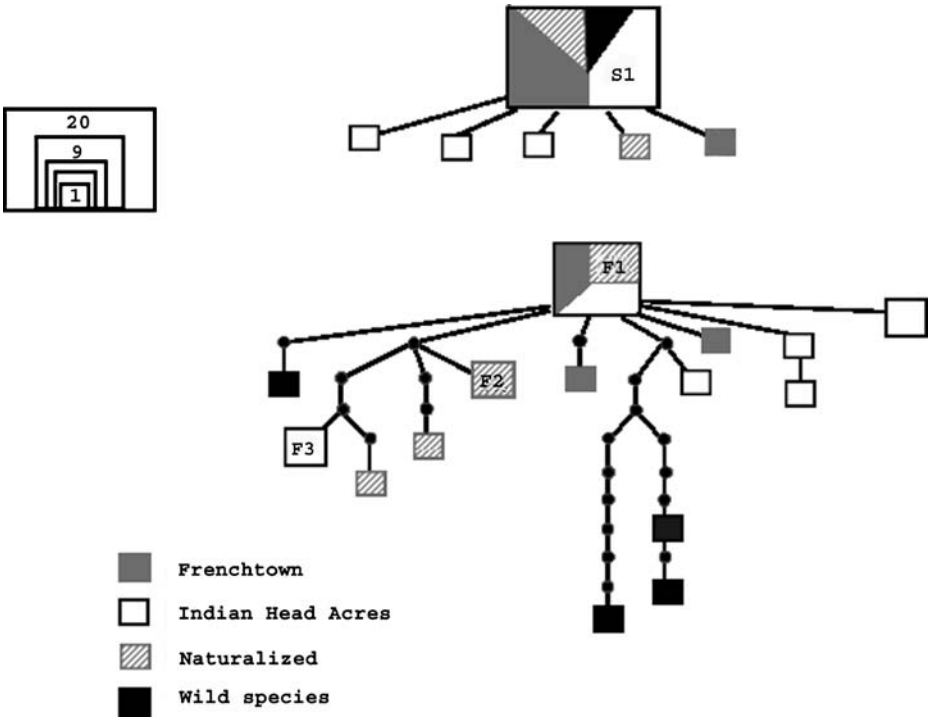

Fig. 4 Haplotype networks for nuclear region 824 of Tallahassee *Wisteria* samples. Top network contains all the *W. sinensis* haplotypes; bottom network are *W. floribunda* haplotypes. Each *branch length* implies a single mutational difference and *black dots* represent unsampled haplotypes. The *size of the circle* is proportional to the number of haplotypes recovered

of hybrid individuals between the two cities is significantly different (Fisher's exact test $p=0.015$); with Tallahassee having more *Wisteria* species than Charleston. When the distribution of hybrid individuals in both cities was divided into the three sample locations (historic, urban expansion and naturalized) no difference in the proportion of hybrid *Wisteria* individuals was found among the different neighborhoods (Fisher's exact test $p=0.34$; Table 2).

Twenty-six of 42 individuals carrying a *W. sinensis* haplotype, whether a hybrid or species, carried the most common *W. sinensis* haplotype (S1). In contrast, the high diversity of *W. floribunda* haplotypes allowed us to measure the association of geographic distance and allelic diversity both within and between the two cities. Regression analysis of the pairwise genetic distance of *floribunda* haplotypes to their physical geographic distance did

Table 2 Identity of *Wisteria* individuals by neighborhood age (not including *W. frutescens*)

	Historic		Urban expansion		Naturalized		Total
	Hybrid	Species	Hybrid	Species	Hybrid	Species	
Charleston	14 (30%)	3 (6%)	18 (38%)	1 (2%)	11 (23%)	0 (0%)	47
Tallahassee	5 (22%)	3 (13%)	7 (30%)	3 (13%)	4 (17%)	1 (4%)	23
Total	19	6	25	4	15	1	70

Row percentages shown

Table 3 Frequency of *floribunda* haplotypes by neighborhood age (cities combined)

	Haplotype F1	Haplotype F2	Haplotype F3	All others	Total
Historic	9 (30%)	4 (13%)	0 (0%)	17 (57%)	30
Urban expansion	6 (17%)	1 (3%)	4 (11%)	22 (63%)	35
Naturalized	7 (32%)	6 (27%)	0 (0%)	11 (50%)	22
Total	22	11	4	50	87

Row percentages shown

not find a significant relationship between the two variables at the within Charleston neighborhoods (R^2=0.010). or naturalized populations (R^2=0.015).

Finally, we wanted to compare the pattern of allelic diversity among the neighborhoods and naturalized populations. A Fisher's exact test of the distribution of shared *floribunda* haplotypes found that the three populations are statistically different (Fisher's exact test, p=0.039; Table 3). This difference is due to the reduced presence of haplotypes F1 and F2 and unique presence of haplotype F3 in the urban expansion neighborhoods. In order to test the distribution of naturalized haplotypes to those in urban neighborhoods, two further tests performed. The first test grouped the naturalized populations with historic neighborhoods while the second grouped the naturalized populations with the 1960s urban expansion neighborhoods. When the naturalized populations are grouped with the urban expansion populations, there is no significant difference between groups (Fisher's exact test, p=0.11); in contrast, when the naturalized populations are grouped with historic populations, this group is significantly different than the urban expansion populations (Fisher's exact test p=0.005).

Discussion

Humans are dependent on plants for food and shelter but our recent access to a diversity of horticultural plants is negatively impacting natural environments. It is estimated that approximately 40,000 plant taxa have been introduced to North America since 1500 A.D.; twice as many species as the native flora (Mack 2005). The majority of species currently imported are introduced for their ornamental value (Reichard and Campbell 1996). The ongoing introduction and diversification of horticultural plant species continues to enhance and beautify our gardens, parks and urban landscapes. Unfortunately, there have been unforeseen impacts of non-native introductions on our natural ecosystems. Urban environments, with the high density of individuals, the great diversity of species and cultivars and the long-term care of dedicated gardeners have become the breeding and trial grounds for the source populations of self-sustaining exotic plant species (Mack 2005). In our study, we have learned that invasive *Wisteria* has a complex history of incorporating both active and passive mechanisms in its invasion history.

Active invasion

Urban environments have created a situation where hybridization acts as an active genetic mechanism to create novel genotypes (Anderson and Stebbins 1954; Arnold 1997). The ecological and genetic impact of hybrids is just beginning to be recognized. We found a higher percentage of hybrid *Wisteria* in naturalized populations than in urban populations (94 vs. 77%), although in our limited sample this difference was not significant. Hybridization

between Chinese and Japanese *Wisteria* species has provided the right combination of genetic diversity and ecological amplitude for *Wisteria* to survive and reproduce in both managed and natural ecosystems in the USA. Further research on the prevalence of hybridization in other urban centers and the biological traits of hybrids will greatly enhance our understanding as to why *Wisteria* hybrids are successful.

In our study of urban *Wisteria* populations, we encountered four *Wisteria* species in Charleston and three in Tallahassee. Three of these four species were found to hybridize together in all combinations. As horticultural interest in *Wisteria* and the related genus *Millettia* increases and these taxa become commercially available, it is likely that further introgression will occur. Our study found that hybridization was restricted to the introduced species of *Wisteria*. The inability of the US native species to hybridize is likely due to its hexaploid chromosome number; the introduced species are all diploids (Valder 1995). If this chromosomal barrier did not exist, the ramifications of hybridization may be even more damaging. Hybridization between ecologically adapted native species could increase the ecological amplitude of the hybrid offspring, enlarging the potential area of impact. In addition, if these native-introduced hybrids had greater fitness and abundance than the native species, the hybrid swarms could lead to the extinction of the natives by reducing the likelihood that native plants would be pollinated by pure native species (i.e. genetic swamping). From this initial assessment, the future of invasive *Wisteria* in the USA can be deduced; as hybridization continues to mix the genetic, phenotypic, and ecological traits of *Wisteria* species, novel combinations will continuously arise to be tested by the environment surrounding them.

The invasion of hybrid *Wisteria* is not a unique phenomenon; there is a growing body of evidence linking hybridization and invasion. Invasive hybrid animals and plants have greatly impacted ecological community structure and function (Daehler and Strong 1997; Gaskin and Schall 2002; Pfenninger et al. 2002; Saltonstall 2002). The mechanisms that increase invasibility through hybridization are still under investigation. One explanation comes from the field of crop breeding. An enormous body of evidence exists for hybrid vigor (heterosis) in hybrid crop plants. Increases in growth rate, size, fruit and seed set have all been attributed to hybridization in crop plants (Bruce 1910; Frankel 1983). Heterosis has been attributed to invasion success in the invasive freshwater snail, *Melanoides tuberculata*. Interspecific hybrids have increased juvenile size and growth rate, allowing them to outcompete their parent species in natural habitats (Facon et al. 2005). Whether *Wisteria* hybrids exhibit heterosis in characteristics important to invasion is currently unknown.

Hybridization is used by plant breeders to introduce desirable traits from one species into another. For example, a hybridization program is attempting to breed rust resistance into soybean (*Glycine max* (L.) Merr.) from its close rust-resistant relative, *Glycine tomentella* Hagata (Singh et al. 1993). Disease resistance has also been transferred by spontaneous hybridization of native and exotic butternut trees. Hybrids of the canker-susceptible *Juglans cinerea* L. with canker–resistant Asian *Juglans ailanthifolia* Carrière show resistance to the pathogen (Michler et al. 2005). Plant invasion has also been linked to the transfer of an adaptive trait through hybridization. The British Isle invasive rhododendron (*R. ponticum* L.) is believed to have gained cold tolerance from hybridization with North American parent *R. catawbiense* Michx. (Milne and Abbott 2000). The natural distribution of *Wisteria sinensis* in China is within the southeastern warm temperate region (Valder 1995). It has been hypothesized that hybridization with cold temperate *Wisteria floribunda* has been necessary for inheritance of cold tolerance in North American hybrid naturalized plants (Valder, personal communication). Although of unknown function, the genetic contribution of *W. sinensis* to the invasive hybrid is evidenced by the overwhelming dominance (88%)

of this species as the maternal parent of hybrid individuals. Our study found that the majority of planted urban *Wisteria* are hybrids and that hybrids are currently sold at major retail garden centers (data not shown). Apparently, hybridization is not simply a key step in the invasion process of *Wisteria* but has also played an important role in the success of horticultural propagation of these plants in the USA.

Passive invasion

Although hybridization of exotic *Wisteria* species has undoubtedly played an important role in its US invasion, our study has found evidence for passive mechanisms as well. Exotic *Wisteria* hybrids in the USA are not a recent phenomenon. Of the sampled hybrids in our study, 93% of individuals belonged to the F2 or greater generation. In long-lived plants such as these, this suggests that hybrids have been around for over half of the nearly 200 years the plants have been in the USA. The relationship of hybridization with time is further supported in the number of hybrids between the two cities. Charleston, one of the oldest southeastern cities, was founded in 1670 while Tallahassee is relatively young, established in 1824. We found significantly fewer hybrid plants in Tallahassee than Charleston, indicating that increasing city age may correlate with an increase in the opportunity for introgression. On a much shorter time scale, time has been found to be important to the genetic identity of naturalized plants, with genotypes in the oldest urban areas more closely related to genotypes of naturalized plants. The relationship of invasion with time is not new; Scott and Panetta (1993) found that a long length of time since introduction (>140 years) was one of the best predictors of invasiveness of southern African plants introduced to Australia.

Interestingly, our study did not find a relationship between density and invasion. The most common *floribunda* haplotype, F1, was encountered 22 times but its distribution was not significantly different between urban and naturalized populations. The most common haplotype is the most likely to be invasive as predicted by a simple model of propagule pressure (Von Holle and Simberloff 2005; Krivánek et al. 2006). In contrast, the second most common haplotype, F2, was statistically more common in naturalized populations. This suggests that this genotype may be actively selected for invasion success although 12 other *floribunda* genotypes were also found in naturalized plants.

Horticulture and invasive plants

One of the major differences between crop agriculture and ornamental plant horticulture is in the degree of inbreeding and level of genetic diversity. Many crop species were domesticated at one time or from a single location and therefore represent just a small subset of the genetic diversity inherent in the wild species (Smith 1998). Ornamental plants gain genetic diversity from a large number of number of introductions from wild populations and to the high proportion of out-crossing species. We found a high level of genetic diversity in ornamental *Wisteria floribunda* taxa with 50 unique haplotypes in 60 individuals. In contrast, we found a lower level of genetic diversity (16 haplotypes in 69 individuals) for *W. sinensis*. This high initial genetic diversity has been increased by hybridization to create a plethora of hybrid *Wisteria* genotypes. Both Chinese and Japanese *Wisteria* have a large number of morphologically distinct cultivars, have a wide distribution in their native lands, are out-breeding and set viable seed. It is speculation to determine why we find lower genetic diversity in Chinese *Wisteria* but the potential lack of cold-tolerant genotypes as mentioned above or a more limited introduction of Chinese cultivars to the western world may play a part.

The role of horticulture and the rapid dispersal of horticulturally propagated cultivars have unified and homogenized the urban and naturalized *Wisteria* populations throughout the southeastern USA. Although *Wisteria* hybrids have a high level of genetic diversity, this diversity is not organized by geographic location. Our regression analyses found that *Wisteria* populations from Charleston and Tallahassee act as one homogeneous population of plants. In addition, our previous study of naturalized *Wisteria* populations found shared haplotypes throughout the southeastern USA (Trusty et al. 2007).

Understanding the importance of human activities and societal expectations is vital in predicting exotic plant invasion worldwide. The economic impact of horticulture, the sociological importance of landscape aesthetics and the role of urbanization and land-use change all affect our ability to predict, respond to and prohibit exotic plant invasions. Human mediated dispersal increases the migration distance and the number of colonization events while urban settlements mix previously isolated floras as well as create a mosaic of managed and unmanaged habitats available to fertile progeny (Ellstrand and Schierenbeck 2000). All of these factors interact to complicate our ability to predict and prevent plant invasion. In the case of invasive exotic *Wisteria*, its long history of horticulture in the USA, high genetic diversity, hybridization between Chinese and Japanese species, and human aided dispersal through horticulture sales are all implicated in the successful ability of these plants to escape gardens and invade natural habitats throughout the USA.

Appendix

List of samples, collection locations, Genbank accession information, and species designations from each genome region; F=floribunda, S=sinensis, H=hybrid. Samples in bold are true species.

City	Location	Chloroplast *trnL-F*	Mitochondria *NAD4*	Nuclear 824	Nuclear 997	Genbank Accession Numbers
Charleston 1	Historic	F	S	F/F	F	EF174043–EF174044
Charleston 2	Historic	S	S	S/S	S	EF174045–EF174046
Charleston 3	Historic	S	S	S/S	S	EF174047–EF174048
Charleston 4	Historic	F	F	F/F	F	EF174049–EF174050
Charleston 5	Historic	F	S	F/F	F	EF174051–EF174052
Charleston 6	Historic	F	S	F/F	H	EF174053–EF174054
Charleston 9	Historic	F	S	F/F	H	EF174055–EF174056
Charleston 10	Historic	F	F	F/F	H	EF174057–EF174058
Charleston 11	Historic	S	S	S/F	S	EF174059–EF174060
Charleston 12	Historic	S	S	S/F	S	EF174061–EF174062
Charleston	Historic	S	S	S/F	S	EF174063–

14						EF174064
Charleston	Historic	S	S	F/F	S	EF174065–
15						EF174066
Charleston	Historic	F	F	S/F	F	EF174067–
16						EF174068
Charleston	Historic	F	S	F/F	F	EF174069–
17						EF174070
Charleston	Historic	S	S	S/F	S	EF174071–
19						EF174072
Charleston	Historic	S	S	S/F	S	EF174073–
20						EF174074
Charleston	Historic	F	S	F/F	F	EF174075–
21						EF174076
Charleston	West Ashley	S	S	S/F	S	EF174077–
22						EF174078
Charleston	West Ashley	F	S	F/F	F	EF174079–
23						EF174080
Charleston	West Ashley	S	S	S/F	S	EF174081–
24						EF174082
Charleston	West Ashley	S	S	F/F	H	EF174083–
25						EF174084
Charleston	West Ashley	S	S	S/F	S	EF174085–
26						EF174086
Charleston	West Ashley	F	S	F/F	H	EF174087–
27						EF174088
Charleston	West Ashley	F	S	F/F	F	EF174089–
28						EF174090
Charleston	West Ashley	S	S	S/F	S	EF174091–
29						EF174092
Charleston	West Ashley	F	S	F/F	H	EF174093–
30						EF174094
Charleston	West Ashley	F	S	S/F	F	EF174095–
31						EF174096
Charleston	West Ashley	S	S	S/F	F	EF174097–
32						EF174098
Charleston	West Ashley	S	S	S/F	S	EF174099–
33						EF174100
Charleston	West Ashley	F	S	F/F	F	EF174101–
34						EF174102
Charleston	West Ashley	S	S	S/S	S	EF174103–
35						EF174104
Charleston	West Ashley	F	S	F/F	F	EF174105–
36						EF174106
Charleston	West Ashley	S	S	S/F	S	EF174107–
37						EF174108
Charleston	West Ashley	F	S	F/F	F	EF174111–
40						EF174112
Charleston	West Ashley	F	S	S/S	F	EF174113–
41						EF174114
Charleston	West Ashley	F	S	S/F	F	EF174115–
42						EF174116
Charleston	West Ashley	S	S	F/F	S	EF174114–
49						EF174118
Charleston	Naturalized	F	S	F/F	F	EF153247–

38						EF153248
Charleston	Naturalized	F	S	S/	H	EF174109–
39				brachybotrys		EF174110
Charleston	Naturalized	F	S	F/F	H	EF153249–
43						EF153250
Charleston	Naturalized	F	S	F/F	S	EF153251–
44						EF153252
Charleston	Naturalized	F	S	S/F	H	EF153253–
45						EF153254
Charleston	Naturalized	S	S	F/	H	EF153261–
46				brachybotrys		EF153262
Charleston	Naturalized	F	S	F/F	S	EF153255–
47						EF153256
Charleston	Naturalized	S	S	S/F	S	EF153227–
48						EF153228
Charleston	Naturalized	S	S	S/F	S	EF153229–
50						EF153230
Charleston	Naturalized	F	F	F/F	H	EF153231–
51						EF153232
Charleston	Naturalized	S	F	S/F	S	EF153233–
52						EF153234
Charleston	Naturalized	S	S	S/F	S	EF153235–
53						EF153236
Tallahasse	Nene	S	S	S/S	S	EF174119–
1						EF174120
Tallahassee	Nene	S	S	S/S	S	EF174121–
2						EF174122
Tallahassee	Nene	S	S	S/F	S	EF174123–
3						EF174124
Tallahassee	Nene	S	S	S/S	S	EF174125–
4						EF174126
Tallahassee	Nene	S	S	S/F	S	EF174127–
5						EF174128
Tallahassee	Nene	S	F	F/F	H	EF174129–
6						EF174130
Tallahassee	Nene	S	S	S/F	S	EF174131–
7						EF174132
Tallahassee	Nene	S	F	F/F	F	EF174133–
8						EF174134
Tallahassee	Nene	S	S	S/F	S	EF174135–
9						EF174136
Tallahassee	Nene	F	F	F/F	H	EF174137–
10						EF174138
Tallahassee	Frenchtown	S	S	S/F	S	EF174139–
11						EF174140
Tallahassee	Frenchtown	S	S	S/F	S	EF174141–
12						EF174142
Tallahassee	Frenchtown	S	S	S/F	S	EF174143–
13						EF174144
Tallahassee	Frenchtown	S	S	S/S	S	EF174145–
14						EF174146
Tallahassee	Frenchtown	S	S	S/F	S	EF174147–
15						EF174148
Tallahassee	Frenchtown	S	S	S/S	S	EF174149–

16						EF174150
Tallahassee 17	Frenchtown	S	S	S/S	S	EF174151–EF174152
Tallahassee 18	Frenchtown	F	S	S/F	S	EF174153–EF174154
Tallahassee 19	Naturalized	S	F	F/F	F	EF153201–EF153202
Tallahassee 20	Naturalized	S	S	S/S	S	EF153203–EF153204
Tallahassee 22	Naturalized	F	S	F/F	H	EF153205–EF153206
Tallahassee 23	Naturalized	F	S	F/F	H	EF153207–EF153208
Tallahassee 24	Naturalized	S	S	S/F	H	EF153209–EF153210
Wisteria sinensis	Kew	S	S	S/S	S	EF153213–EF153214
Wisteria sinensis	Lathrop	S	S	S/S	S	EF174155–EF174156
Wisteria floribunda	Kew	F	F	F/F	F	EF153219–EF153220
Wisteria floribunda	Arnold Arboretum	F	F	F/F	F	EF153221–EF153222

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