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Patterns of hybridization and introgression between invasive *Ulmus pumila* (Ulmaceae) and native *U. rubra*[1]

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Natural hybridization between introduced species and their native congeners occurs frequently and can create serious conservation concerns. *Ulmus pumila* (Siberian elm) is an introduced Asian elm species that has naturalized in the United States and is now considered invasive in 41 states. Red elm (*U. rubra*), a native to the eastern United States, often occurs in sympatry with Siberian elm, and the two species are thought to hybridize. Here, we genetically characterized reference populations of the two elm species to identify species-specific microsatellite alleles. These markers were used to classify individuals in putative hybrid zones as parental species or hybrids, assess the extent of hybridization, and track patterns of introgression. We identified nine *U. rubra*, 32 *U. pumila*, and 51 hybrid individuals in our hybrid zones. Of the 51 hybrids, 35 were classified as first-generation hybrids and 16 as backcrosses. The majority of the backcrosses (88%) were introgressed toward *U. pumila*. Our classification of genotypes was consistent whether we used manual classification, principal coordinate analyses or Bayesian clustering. We observed greater genetic diversity and new combination of alleles in the hybrids. Our study indicates widespread hybridization between *U. pumila* and *U. rubra* and an asymmetric pattern of introgression toward *U. pumila*.

Key words: Dutch elm disease; hybrid zones; invasive species; microsatellites; red elm; Siberian elm; species-specific markers.

The introduction of plant species into new areas can lead to significant ecological and genetic changes in both the introduced and native species (Strauss et al., 2006). Several recent studies have documented that natural hybridization between introduced species and their native congeners occurs frequently and can create serious conservation concerns (Ellstrand and Schierenbeck, 2000; Rieseberg et al., 2003). Continued hybridization may result in the genetic assimilation and eventual loss of native taxa (Rhymer and Simberloff, 1996; Hedge et al., 2006). This genetic swamping can be especially detrimental for small populations already at risk from biotic or abiotic stresses (Rieseberg et al., 1989; Ellstrand and Elam, 1993; Daehler and Strong, 1997; Collin, 2002; Burgess et al., 2005; Prentis et al., 2007). Hybridization between species may also stimulate the evolution of invasiveness by increasing genetic diversity and creating new genotypes (Ellstrand and Schierenbeck, 2000; Sakai et al., 2001; Hedge et al., 2006). In fact, hybrid zones represent regions of unparalleled genetic variation and unique gene combinations where selection may be intense and evolution rapid (Keim et al., 1989). In addition, the conservation problems posed by hybridization may neither be restricted to species at risk nor only to those species that hybridize (Vila et al., 2000), but could threaten entire ecosystems as a result of the evolution of exotic "super invasive" species (Londo and Schaal, 2007; Moody and Les, 2007; Wolfe et al., 2007).

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During the 20th century, two Dutch elm disease (DED) pandemics caused by the fungi *Ophiostoma ulmi* and *O. novo-ulmi* decimated populations of native elms worldwide (Brasier, 1988, 1991). Because of their high degree of susceptibility to DED, European and North American native elm species have been particularly affected, with infected trees usually dying within 1–2 years (Smalley and Guries, 1993). As a result of the loss of native elm populations by DED and the subsequent ecological changes in wild and urban forests, the conservation of elm genetic resources has become a major concern, especially in Europe (Machon et al., 1997; Cogolludo-Agustin et al., 2000; Goodall-Copestake et al., 2005). In contrast, several Eurasian elm species possess varying degrees of tolerance and can survive and even thrive with DED (Smalley and Guries, 1993). For example, Siberian elm (*Ulmus pumila* L.; $2n = 2x = 28$), native to East Asia, is highly adaptable and tolerant to DED (Smalley and Guries, 1993). Siberian elm was introduced in the United States beginning about 1900 prior to the first DED pandemic (Ware, 1995). Since that time, Siberian elm has naturalized, and it is now considered a noxious weed or invasive species in 41 states (USDA, NRCS, 2002; Ding et al., 2006). Currently, Siberian elm poses a dilemma; it has been the source of the DED resistance genes in virtually every cultivar released in the United States during the past 30 years (Smalley and Guries, 1993), yet it has the potential to be an aggressive invader of disturbed areas throughout North America (Ding et al., 2006).

The ability of *U. pumila* to cross-hybridize with different elm species has been extensively documented (Santamour, 1972; Townsend, 1975) and exploited to develop DED tolerant hybrids with susceptible species (Smalley and Guries, 1993; Ware, 1995; Mittempergher and Santini, 2004). However, hybridization in the wild between naturalized *U. pumila* and native elm species is largely unrecognized and poorly understood (Cogolludo-Agustin et al., 2000). Studies assessing natural hybridization in *Ulmus* are important because genetic barriers for interspecific hybridization are few, and such studies may help

clarify the evolution and current taxonomic problems in the genus (Wiegrefe et al., 1994). Moreover, recent studies using isozymes, ISSR, and RAPD have demonstrated that natural hybrids between *U. pumila* and *U. carpinifolia* occur frequently in Europe (Cogolludo-Agustin et al., 2000; Goodall-Copestake et al., 2005). However, such studies have been limited by the low level of polymorphisms in isozymes and the inefficiency of dominant markers to address the issue of introgression. More recently, highly informative microsatellite markers have been developed for European (*U. laevis*, Whiteley et al., 2003; *U. carpinifolia*, Collada et al., 2004) and North American (*U. rubra*, Zalapa et al., 2008a) elm species. Cross-amplification tests (Whiteley et al., 2003; Collada et al., 2004; Zalapa et al., 2008a, b) in several elm species suggest the potential broad applicability of such microsatellite markers for hybridization and introgression studies in the genus *Ulmus*.

Red elm (*Ulmus rubra* Mulh.; $2n = 2x = 28$), a native to the eastern United States (USDA, NRCS, 2002), often occurs in sympatry with Siberian elm, and the two species are thought to commonly hybridize wherever plantings of Siberian elm are in close proximity to wild populations of red elm (Lester and Smalley, 1972a, b). However, no genetic studies have been conducted to confirm the existence or measure the extent of natural hybridization or introgression between these two elm species. In addition, we do not know whether introgression is bilateral or unilateral toward one of the parental species and whether hybridization increases levels of genetic diversity. This knowledge may help us determine whether hybridization preceded and contributed to the level of invasiveness observed in the Siberian elm throughout the United States. Also, because red elm is highly susceptible to DED, and natural populations have been severely decimated (Lester and Smalley, 1972a, b), Siberian elm may not only outcompete, but genetically assimilate red elm (Rhymer and Simberloff, 1996). Thus, the goals of this study were to (1) genetically confirm the existence of natural hybridization between *U. rubra* and *U. pumila*; (2) assess any evidence of introgression to either parental species, and (3) compare the level of genetic diversity between hybrids and parental species. This research provides an evaluation and discussion of hybridization and its effects in exotic, invasive *U. pumila* and native *U. rubra* in the context of the current DED pandemics. This study is one of the first to examine the impact of germplasm from an introduced species on the conservation of a wild native tree species. Moreover, because natural hybridization is likely to occur between *U. pumila* and other elm species both in Europe and the United States (Santamour, 1972; Townsend, 1975; Cogolludo-Agustin et al., 2000; Goodall-Copestake et al., 2005; Zalapa et al., 2008a), our research provides a model for the study of natural hybridization in the genus *Ulmus*.

MATERIALS AND METHODS

Sampling of plant materials—To identify hybrids, we first determined the genetic composition of pure reference populations of *U. pumila* and *U. rubra*. The following criteria were used to identify and distinguish the two species in the field. Siberian elm possesses symmetrical, once-serrate, small leaves (3–7 cm long); slender, smooth, hairless twigs; small, blunt, hairless buds; shallowly furrowed, gray or brown bark; and comparatively small, smooth samaras (Wyman, 1951). In contrast, red elm can be readily distinguished from other elms in Wisconsin by its large (10–18 cm long), sharply double serrated, scabrous leaves; scabrous-pubescent twigs; comparatively large, red hair-covered winter-buds; reddish, deeply furrowed bark; and nearly round, hairless (margin and surface) samaras (Wyman, 1951).

In Wisconsin, *U. pumila* trees were planted adjacent to highways and farms and close to villages and towns to serve as wind breaks, and in urban landscapes to replace dead or dying DED-susceptible native elms (Ware, 1995). However, phenotypically pure (Wyman 1951), naturalized *U. pumila* populations were difficult to locate in Wisconsin. Therefore, to properly identify species-specific markers and to establish a genetic profile for *U. pumila* in East Asia, we used genotypic data from 53 accessions collected across the Peoples' Republic of China (PRC) and maintained at the University of Wisconsin (UW) Elm Arboretum in Arlington, Wisconsin (Zalapa et al., 2008b). In contrast, phenotypically pure (Wyman 1951) *U. rubra* populations, although sparse, were relatively easy to identify in Wisconsin. We collected 100 *U. rubra* tree samples at five sites (20 individuals/site; sites ~30–200 km apart; Table 1). The five collection sites were chosen because they represented relatively large (12–1202 ha) forest parcels containing *U. rubra* populations in relative isolation from *U. pumila*. In addition, 25 *U. rubra* herbarium specimens, originally collected between 1890–1965 (20 specimens collected before 1960; UW-Herbarium, Madison, Wisconsin; Appendix 1), were sampled for comparative assessment of historic Wisconsin statewide genetic resources. Because *U. pumila* was not widely planted in Wisconsin until the late 1930s (Ware, 1995) and *U. pumila* herbarium records start in 1938 with only 12% of them recorded before 1960, we expect that our *U. rubra* herbarium specimens will represent the genetic variation of the species preceding the natural spread of *U. pumila*. Finally, in addition to the reference populations, we collected 95 individuals from six Wisconsin locations (7–20 individuals/location; Table 1) in which several trees had leaf, bark, twig, and/or seed characteristics intermediate between *U. pumila* and *U. rubra*.

Microsatellites and genetic characterization of parental species—Leaves from each tree were freeze-dried for 72 h using a BenchTop lyophilizer (Virtis, Gardiner, New York, USA). DNA was extracted using a DNeasy kit (Qiagen, Valencia, California, USA), and concentrations were measured in a Turner Quantech fluorometer (Barnstead, Dubuque, Iowa, USA). We used nine microsatellite primer pairs (UR123, UR141, UR153, UR158, UR159, UR173a, UR188a, ULM-2, and ULMI-98), previously characterized in *U. rubra* ($N = 20$ individuals; Zalapa et al., 2008a) and cross-amplified in *U. pumila* (Zalapa et al., 2008b), which amplified specific alleles for each of the two species. Using 16 randomly selected putative hybrid individuals, we first tested the microsatellite markers to determine their ability to detect hybrid progeny and consistency (i.e., repeatability) of amplification patterns in the subset of putative hybrids. Loci were then used to develop a diagnostic genetic profile for each parental species. We used a comparative approach to characterize the two parental species and evaluated (1) native *U. rubra* (RU) populations without apparent contact with *U. pumila* (100 trees, 20 from each of five populations); (2) representative accessions from *U. rubra* (RU) populations across Wisconsin (25 historic herbarium specimens); and (3) representative accessions from *U. pumila* (PU) populations in their native range (53 accessions from PRC) (Zalapa et al., 2008b).

PCRs were performed in 15 μL total volume using 1.5 μL 10× PCR buffer, 1.8 μL 25mM $MgCl_2$, 2.4 μL dNTPs (1.25 mM of each dATP, dGTP, dTTP, and dCTP), 1.0 μL 5 μM primer, 2 μL 10 ng/μL genomic DNA, 1 U *Taq* DNA polymerase (Lucigen, Middleton, Wisconsin, USA), and 6.2 μL H_2O. Thermocycling conditions were as follows: an initial melting step (94°C for 3 min), then 30 cycles (94°C for 15 s, 55°C/60°C for 90 s, and 72°C for 2 min), and a final elongation step (72°C for 20 min), and then an indefinite soak at 4°C. Microsatellite allele genotyping using fluorescent labeled primers (5′ end 6-FAM [6-carboxyfluorescein] fluorophore; IDT Coralville, Iowa, USA) was performed at the UW Biotechnology Center DNA Sequence Facility using an ABI 3730 fluorescent sequencer (POP-6 and a 50 cm array; Applied Biosystems, Foster City, California, USA) and a Gensize Rox 650 ladder (GENPAK Ltd., Brighton, UK). Alleles were scored using GeneMarker Software version 1.5 (SoftGenetics, State College, Pennsylvania, USA). PCR reactions were repeated periodically to ensure the repeatability of the results.

Identification of parental species and hybrids in the contact zones—Using the genetic profiles developed for each parental reference population, we manually classified the 95 individuals collected from the six locations forming contact zones between *U. rubra* and *U. pumila* as either *U. rubra* individuals [RU(HY)], *U. pumila* individuals [PU(HY)], or hybrid individuals [HY(HY)]. Furthermore, we categorized confirmed hybrid individuals as first-generation hybrids [F_1(HY)] or backcrosses to one of the parental species [B_C(HY)]. A first-generation hybrid individual was heterozygous for species-specific alleles at all loci; a backcross individual had at least one locus fixed for species-specific alleles for one of the two parental species, while all other loci were

TABLE 1. Wisconsin elm taxa, collection sites, and number of samples for the study of hybridization.

Taxon	Collection sites	Location (County)	Latitude, longitude	*N*
Ulmus rubra (RU)				125
	UW-Madison Arboretum	Madison (Dane)	42.02°N, 89.25°W	20
	Hall's Woods	Brooklyn (Green)	42.48°N, 89.24°W	20
	Wisconsin Riverway	Troy (Sauk)	43.12°N, 89.57°W	20
	Coulee Experimental Forest	Bangor (La Crosse)	43.51°N, 91.01°W	20
	Baraboo Hills	North Freedom (Sauk)	43.24°N, 89.54°W	20
	UW-Herbarium	Madison (Dane)		25
Ulmus pumila (PU)				53
	UW-Elm Arboretum	Vienna (Dane)		53
Putative hybrid (HY)				95
	Shady Lane	Wisconsin Dells (Columbia)	43.32°N, 89.46°W	20
	Dove Tail	Burke (Dane)	43.08°N, 89.21°W	20
	County Ab	Blooming Grove (Dane)	43.04°N, 89.15°W	20
	Irish Lane	Fitchburg (Dane)	42.59°N, 89.22°W	20
	Burve	Deerfield (Dane)	43.05°N, 89.07°W	8
	MM	Fitchburg (Dane)	42.57°N, 89.22°W	7

heterozygous for the species-specific alleles. The thorough characterization of species-specific variability in reference parental populations before the examination of hybrids permitted an unambiguous classification of the individuals collected in the putative hybrid populations.

We conducted a principal coordinate analysis (PCoA) on the genetic data to summarize major patterns of variation in the multilocus data set. The PCoA was based on genetic distances estimated between pairs of individuals (Smouse and Peakall, 1999) and was computed using the program GeneAlEx version 6.0 (Peakall and Smouse, 2006). We also used the program STRUCTURE version 2.2, a Bayesian model-based clustering method to assign individuals to K populations based on their multilocus genotypes (Pritchard et al., 2000). This program calculates an admixture coefficient (q) for each genotyped individual, where q represents the proportion of an individual's genotype that originates from each reference population (i.e., degree of ancestry). With two parental species, first-generation hybrids (F_1) are expected to have a q value of 0.5, while q values for parental species are expected to be closer to 1. We used the genetic admixture analysis of the program STRUCTURE and an a priori model assumption of $K = 2$ to account for the two parental species. In addition, we selected the option of correlated allele frequencies, a burn-in period of 50 000 steps, and 100 000 Markov chain Monte Carlo (MCMC) replicates; each run was replicated five times to ensure consistency of results.

Parental species and hybrid genetic diversity—We examined the genetic diversity within each of the two parental species and the putative hybrids. Deviations from Hardy–Weinberg equilibrium (HWE) for each locus, and the linkage disequilibrium between loci were estimated using the program POPGENE version 1.3. We used GeneAlEx 6 (Peakall and Smouse, 2006) to describe the observed (A_o) and effective (A_e) number of alleles, the number of alleles with frequency greater than 0.05 (A_o freq > 0.05), the levels of observed (H_o) and expected (H_e) heterozygosity, and the Shannon's information index (I). After describing the genetic diversity within each group, we examined the degree of genetic differentiation among groups. First, we compared the levels of genetic differentiation within and among the three groups using an analysis of molecular variance (AMOVA) (Excoffier et al., 1992). Second, we examined genetic differentiation among groups by calculating pairwise F_{st} using GeneAlEx 6 values between our two parental species (RU and PU) and the hybrids [HY(HY)], as well as between all types of individuals in the putative hybrid zones [RU(HY), PU(HY), F_1(HY), and B_C(HY)].

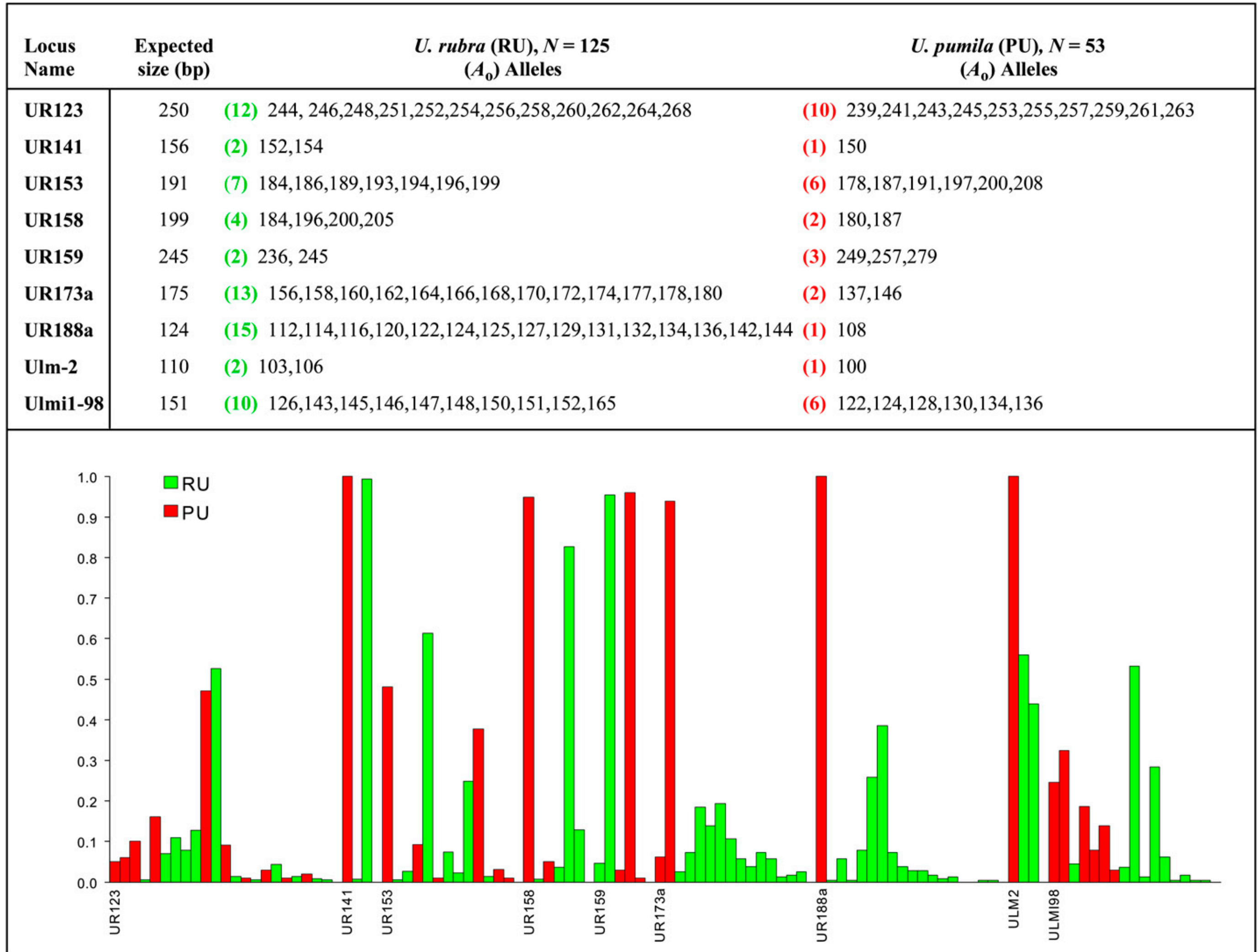
RESULTS

Microsatellites and genetic characterization of parental species—All nine primers (UR123, UR141, UR153, UR158, UR159, UR173a, UR188a, ULM-2, and ULMI-98) amplified clearly in both parental species (Fig. 1) and in the subset of 16 putative hybrids. Amplification products of all genotyped individuals from each microsatellite locus corresponded to the expected lengths (Zalapa et al., 2008a). We detected no significant differences in the microsatellite allelic patterns (i.e., number of alleles with frequency greater than 0.05; Appendix 2) between *U. rubra* populations collected in Wisconsin and *U. rubra* herbarium specimens, and thus we pooled these data (hereafter denominated RU). We detected 99 alleles, whereby 67 alleles were specific to *U. rubra*, and 32 alleles were specific to *U. pumila* (Fig. 1; Appendix 2). In *U. rubra* all nine loci were polymorphic while only six loci were polymorphic in *U. pumila* (Fig. 1; Appendix 2). Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (data not presented) were not statistically significant ($P > 0.05$), except for UR173a, which was not in HWE in PU ($P < 0.001$).

Identification of parental species and hybrids in the contact zones—Of the 95 individuals collected in putative hybrid populations, nine were identified as genetically pure *U. rubra* genotypes [RU(HY)], 32 were classified as pure *U. pumila* genotypes [PU(HY)] and 51 possessed species-specific alleles from each of the two parental species at one or more loci [HY(HY)] and thus were identified as hybrids (Table 2; Appendix 2). Three individuals failed to PCR-amplify after repeated attempts. Among the 51 hybrids, 35 individuals were further classified as first-generation hybrids [F_1(HY)], and 16 individuals were classified as putative backcrosses [B_C(HY)]. Because backcross individuals were relatively few, we did not further subdivide them except to identifying backcrosses to either *U. pumila* (N = 14) or *U. rubra* (N = 2).

A total of 75 alleles were detected in the putative hybrid (HY) populations, including 46 RU alleles and 27 PU alleles, and two alleles from locus UR188a that were not detected in either of the parental reference populations, but likely came from RU since PU is monomorphic at this locus (Appendix 2). The nine RU(HY) individuals had a total of 28 RU alleles, the 32 PU(HY) individuals had 25 PU alleles, and the 51 HY(HY) individuals had 63 alleles, of which 42 were from RU and 21 were from PU.

Principal coordinate analysis and structure—Principal coordinate analysis (PCoA) using the 67 RU and 32 PU species-specific alleles clearly separated *U. rubra* and *U. pumila* from the hybrids (Fig. 2). The first principal coordinates accounted for 86.9% of the genetic variance and separated the two parental species [PU/PU(HY) and RU/RU(HY)] from the hybrids [i.e., HY(HY), which included F_1(HY) and B_C(HY)]. The second



Locus Name	Expected size (bp)	*U. rubra* (RU), *N* = 125 (A_o) Alleles	*U. pumila* (PU), *N* = 53 (A_o) Alleles
UR123	250	**(12)** 244, 246,248,251,252,254,256,258,260,262,264,268	**(10)** 239,241,243,245,253,255,257,259,261,263
UR141	156	**(2)** 152,154	**(1)** 150
UR153	191	**(7)** 184,186,189,193,194,196,199	**(6)** 178,187,191,197,200,208
UR158	199	**(4)** 184,196,200,205	**(2)** 180,187
UR159	245	**(2)** 236, 245	**(3)** 249,257,279
UR173a	175	**(13)** 156,158,160,162,164,166,168,170,172,174,177,178,180	**(2)** 137,146
UR188a	124	**(15)** 112,114,116,120,122,124,125,127,129,131,132,134,136,142,144	**(1)** 108
Ulm-2	110	**(2)** 103,106	**(1)** 100
Ulmi1-98	151	**(10)** 126,143,145,146,147,148,150,151,152,165	**(6)** 122,124,128,130,134,136

Fig. 1. Species-specific microsatellite alleles and frequency distributions for nine microsatellite loci from *Ulmus rubra* and *U. pumila*. A_o = observed number of alleles; RU = Wisconsin *U. rubra* reference individuals (populations + herbarium); PU = Chinese *U. pumila* reference individuals.

TABLE 2. Genetic diversity characteristics for *Ulmus rubra*, *U. pumila*, and putative hybrids based on species-specific allelic variation at nine microsatellite loci. Allele frequencies, *N* = number of individuals; A_o = observed average number of alleles per locus, *A* freq. > 5% = mean number of alleles with frequency greater than 0.05 per locus; A_e = average effective number of alleles per locus; *I* = Shannon index of diversity; H_o = average observed heterozygosity per locus; and H_e = average expected heterozygosity per locus.

	Parental and putative hybrids			Hybrid identification			Hybrid class	
Multilocus means	RU	PU	HY	RU(HY)	PU(HY)	HY(HY)	F_1(HY)	B_C(HY)
N	125	53	92	9	32	51	35	16
A_o	7.44	3.56	8.33	3.22	2.78	7.22	7.00	5.44
A freq. > 5%	3.33	2.44	3.56	3.22	2.22	4.11	4.00	3.67
A_e	2.89	1.89	3.30	2.38	1.83	3.48	3.48	3.25
I	1.07	0.56	1.32	0.83	0.50	1.37	1.38	1.27
H_o	0.49	0.25	0.63	0.52	0.26	0.90	1.00	0.69
H_e	0.49	0.27	0.63	0.45	0.26	0.67	0.67	0.64

Note: RU = Wisconsin *U. rubra* individuals (populations + herbarium); PU = Chinese *U. pumila* individuals; HY = all individuals from putative hybrid populations; RU(HY) = *U. rubra* individuals from contact zones; PU(HY) = *U. pumila* individuals from contact zones; HY(HY) = all confirmed hybrid individuals from contact zones; F_1(HY) = confirmed first-generation hybrids; B_C(HY) = confirmed backcrossed hybrids.

coordinate accounted for 3.7% of the variance and allowed the differentiation of individuals within each of the parental species and within the hybrids. Interestingly, the estimated genetic differences among individuals within a species were much greater for *U. rubra* and the hybrids than for *U. pumila* (Fig. 2). Many of the hybrid individuals (mainly first-generation hybrids)

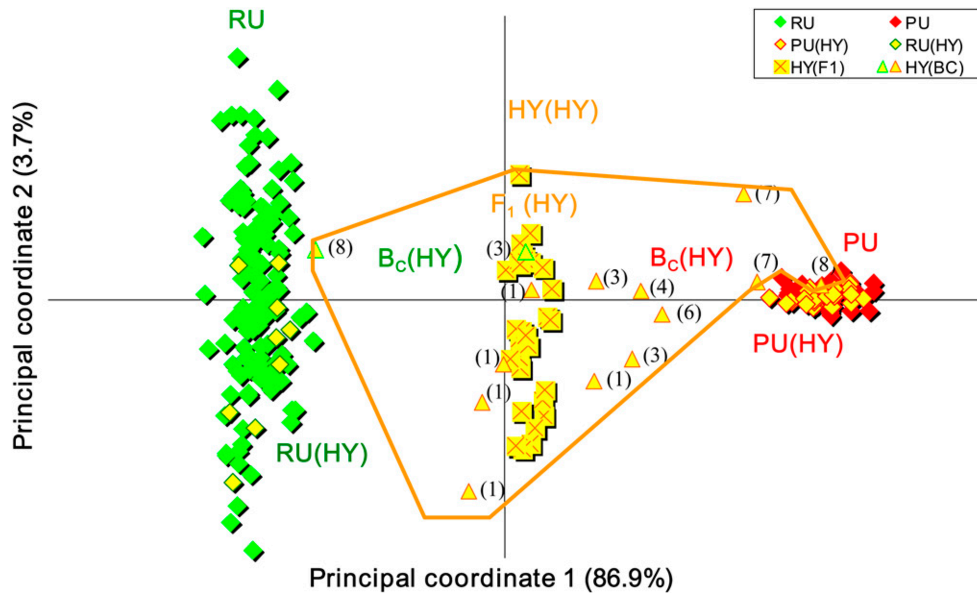


Fig. 2. Principal coordinate analysis for *Ulmus rubra*, *U. pumila*, and putative hybrids. The percentage of the total variation in the data set that is explained by each principal coordinate is given in parentheses. RU = Wisconsin *U. rubra* reference individuals (populations + herbarium); PU = Chinese *U. pumila* reference individuals; HY = all individuals from putative hybrid populations; RU(HY) = *U. rubra* individuals from contact zones; PU(HY) = *U. pumila* individuals from contact zones; HY(HY) = all confirmed hybrid individuals from contact zones; F_1(HY) = confirmed first-generation hybrids; B_C(HY) = confirmed backcrossed hybrids; (#) = number of introgressed loci fixed for a parental species.

appeared in the middle of the PCoA between the two parental species, although there was a biased pattern of introgression back toward *U. pumila* In the hybrid populations, we detected backcross individuals with 1–8 loci fixed for *U. pumila* species-specific alleles.

Using the software STRUCTURE in admixture analysis mode, with $K = 2$ to represent the parental species, all individuals that we had manually identified as *U. rubra* were assigned to *U. rubra* ($q = 0.996$; Fig. 3). These individuals included 125 individuals collected from reference *U. rubra* populations (RU) and nine individuals from the hybrid populations [RU(HY)]. Similarly, all individuals manually identified as *U. pumila* were assigned to *U. pumila* ($q = 0.996$). These included the 53 accessions originally collected from the PRC (PU) and 32 individuals from the hybrid populations [PU(HY)]. The 35 individuals manually classified as first-generation hybrids assigned equally to either of the two parental species ($q = 0.5$) while, as expected, a wide range of admixture proportions were observed for the backcross individuals (Fig. 3). These admixture proportions corresponded to the number of loci fixed for specific alleles from the parental species to which the hybrid presumably backcrossed. These results confirm the presence of genetic hybrids between *U. pumila* and *U. rubra* and support the differential pattern of introgression toward the exotic, invasive species *U. pumila*.

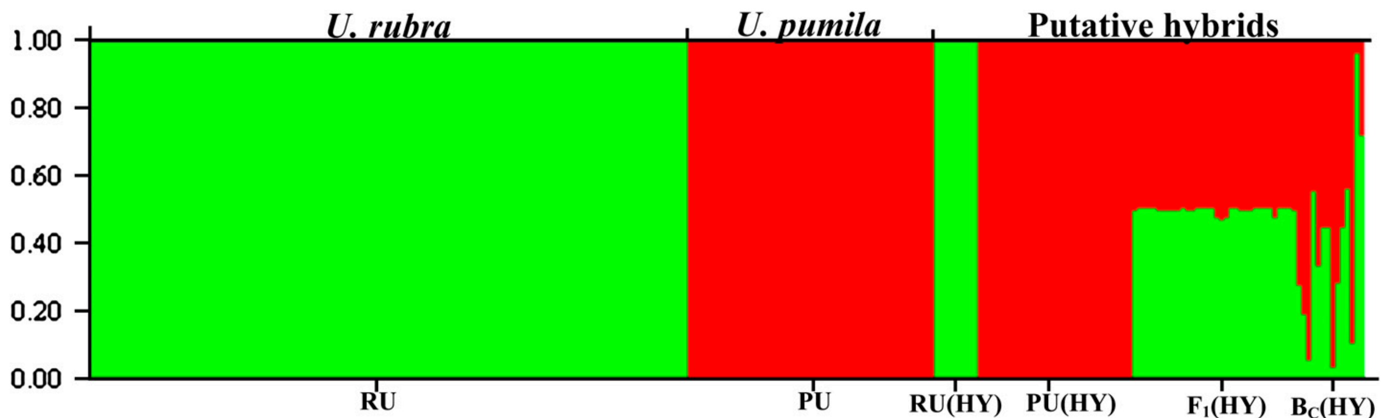


Fig. 3. Clustering results ($K = 2$) for *Ulmus rubra* (RU; green) and *U. pumila* (PU; red) reference populations and confirmed pure individuals from each species and hybrids from Wisconsin contact zones. Each individual is represented as a vertical line partitioned into colored segments, the length of which is proportional to the individual's estimated *K* cluster membership coefficients. RU = Wisconsin *U. rubra* reference individuals (populations + herbarium); PU = Chinese *U. pumila* reference individuals; HY = all individuals from putative hybrid populations; RU(HY) = *U. rubra* individuals from contact zones; PU(HY) = *U. pumila* individuals from contact zones; HY(HY) = all confirmed hybrid individuals from contact zones; F_1(HY) = confirmed first-generation hybrids; B_C(HY) = confirmed backcrossed hybrids.

Parental species and hybrid genetic diversity—The observed mean number of alleles per locus was higher in the *U. rubra* (RU = 7.4 alleles) and hybrid populations (HY = 8.3 alleles) relative to the *U. pumila* populations (PU = 3.6 alleles) (Table 2). When we excluded the individuals classified as *U. rubra* and *U. pumila* from the contact zones, we detected an average of 7.2 alleles per locus in the population of hybrid individuals [HY(HY)]. For *U. rubra* and the hybrids, approximately 50% of the alleles were present at low frequency (<0.05), while 68% of the alleles in *U. pumila* were present at low frequency. As expected, there was an excess of heterozygotes in the hybrid populations, which we can attribute mainly to the presence of F_1 individuals [F_1(HY)]. Moreover, the level of heterozygosity was greatest in the hybrids, followed by *U. rubra* and lowest in *U. pumila*. Similarly, the hybrid populations had the highest level of gene diversity (*I*), and *U. pumila* populations had the lowest (1.32 vs. 0.56).

Analysis of molecular variance (AMOVA) indicated that 57% of the genetic variation was found among the three groups [RU, PU and HY(HY)], although there was still significant variation within groups (43%). The degree of genetic differentiation was large between *U. rubra* and *U. pumila* (pairwise F_{ST} = 0.5; Table 3). The degree of genetic differentiation was similar between the hybrids [HY(HY)] and *U. rubra* (RU) or *U. pumila* (PU) (pairwise F_{ST} = 0.153 and 0.164, respectively). The degree of genetic differentiation among the other group pairs followed expectations.

DISCUSSION

The first goal of this study was to genetically confirm the occurrence of hybridization between naturalized *U. pumila* and native *U. rubra*. To this end, we first described the genetic profiles of relatively large reference populations of *U. rubra* (*N* = 125) and *U. pumila* (*N* = 53) to confirm the presence of species-specific markers on a larger scale than previously described (Zalapa et al., 2008a). The reference population for *U. pumila* was based on trees grown from seeds originally collected from China (Zalapa et al., 2008b), thus having no possibility of contamination with *U. rubra* alleles. For *U. rubra*, we did not find differences in genetic profile between historic herbarium specimens (mostly collected prior to 1960) and our current samples from wild *U. rubra* populations. This fact strengthened our notion that we had collected pure *U. rubra* populations based on our phenotypic assessment in the field. Moreover, the fact that out of 75 alleles detected in the putative hybrids, only two non-parental alleles (from locus UR188a) were detected, confirmed that our genetic profiles had identified the majority of species-specific alleles for both *U. pumila* and *U. rubra* (Appendix 2).

The nine sets of microsatellite primer pairs consistently discriminated between *U. pumila* and *U. rubra* and identified their hybrid progeny in contact zones. Furthermore, the primers allowed the detection of first-generation hybrids (F_1) as well as backcrosses to each of the two parental species. Discrimination between parental species and the different types of hybrids was consistent whether we manually compared the individuals' genetic profiles, used principal coordinates analyses, or employed the software STRUCTURE (Pritchard et al., 2000) to assign individuals to genetic clusters (in this case, species). A minimum of two microsatellite loci (UR123 + UR153 or Ulmi1–98) were sufficient to identify up to 96% of the hybrids manually, although the entire set was required for classification of advanced hybrids (i.e., B_C progeny). The putative hybrid zones contained 51 confirmed hybrid individuals (55%), demonstrating a high incidence of hybridization. Such high proportion of hybrid progenies in the contact zones is consistent with the high rates of hybridization detected in Europe between *Ulmus carpinifolia* (syn. *U. minor*) and naturalized *U. pumila* using isozymes (Cogolludo-Agustin et al., 2000) and dominant DNA markers (RAPD and ISSR; Goodall-Copestake et al., 2005). Moreover, the preponderance of F_1 hybrids (35 of 51 individuals) in the hybrid zones, combined with the fact that elms have a generation time of 10–20 years, suggests recent hybridization events. While our study confirmed that *U. rubra* and *U. pumila* are genetically well-differentiated species (Wiegrefe et al., 1994), the extensive formation of hybrids supports a lack of reproductive isolation mechanisms between the two species. In fact, the absence of crossing barriers between various elm species has been exploited by breeders attempting to introduce DED tolerance to North American and European elms from disease-tolerant Asian species, including *U. pumila* (Smalley and Guries, 1993; Mittempergher and Santini, 2004). Our data indicate that the two species overlap in their geographic distribution and spring flowering phenologies and that wind-dispersed pollen grains from these two elm species frequently land on the stigmas and successfully fertilize ovules of one another. Thus, our study genetically confirms the presence of hybrids between *U. rubra* and *U. pumila* leading to the common and widespread hybridization of these elm tree species in natural and disturbed landscapes.

The second goal of this study was to determine whether introgression occurred in the hybrid zones and whether it was bilateral or unilateral toward one of the parental species. The presence of various degrees of backcrossing indicates that

Table 3. Pairwise genetic differentiation (F_{ST} values) among *Ulmus rubra*, *U. pumila*, and putative hybrids based on species-specific allelic variance at nine microsatellite loci.

Population	RU	PU	HY	RU(HY)	PU(HY)	HY(HY)	F_1(HY)	B_C(HY)
RU	0							
PU	0.500	0						
HY	0.214	0.112	0					
RU(HY)	0.032	0.528	0.232	0				
PU(HY)	0.499	0.018	0.112	0.528	0			
HY(HY)	0.153	0.164	0.008	0.172	0.165	0		
F_1(HY)	0.139	0.179	0.013	0.159	0.179	0.001	0	
B_C(HY)	0.198	0.127	0.004	0.218	0.130	0.007	0.013	0

Notes: RU = Wisconsin *U. rubra* individuals (populations + herbarium); PU = Chinese *U. pumila* individuals; HY = all individuals from putative hybrid populations; RU(HY) = *U. rubra* individuals from contact zones; PU(HY) = *U. pumila* individuals from contact zones; HY(HY) = all confirmed hybrid individuals from contact zones; F_1(HY) = confirmed first-generation hybrids; B_C(HY) = confirmed backcrossed hybrids.

hybridization has occurred over several elm generations in Wisconsin, probably beginning in the 1950s because *U. pumila* was widely planted in the state in the 1930s and can take 10–20 years to reach maturity (Smalley and Guries, 1993; Ware, 1995). The rarity of self-fertility in elms (Mittempergher and Santini, 2004) supported the identification of the 16 individuals (17%; 1–8 loci fixed for alleles specific to one or the other of the two parental species) as true backcrosses. In addition, we found no individuals possessing species-specific homozygous loci from both species, a pattern that would be expected from selfing or crosses between F_1 hybrids. This further supported high rates of backcrossing and in particular a pattern of recurrent backcrosses toward one of the parental species, *U. pumila*. A lack of F_1 hybrid crosses could result from pollen–stigma incompatibilities between F_1 hybrid parents. Self-incompatibility is a common phenomenon in *Ulmus* (Santini et al., 2008), so incompatibilities in F_1 hybrid crosses may be likely. In fact, while each species may have its own S alleles and be likely to cross with each other, F_1 individuals would have S alleles from both species and thus be much less likely to successfully cross with other F_1 individuals. An alternative explanation is that crosses between F_1 hybrids occur but that individuals containing homozygous loci from both species are selected against. This explanation suggests that segments of the genome containing combination of homozygous loci from both parents are not favored and not maintained in the hybrids. It is known that hybrids formed in the absence of chromosomal duplication (homoploid hybrids) can have substantial genomic alterations and that synergistic interactions of alleles at heterozygous loci may confer hybrid vigor (Baack and Rieseberg, 2007). However, one must still explain why individuals with homozygous loci from both parents rather than from one of the parents would be at a greater disadvantage. We favor the simpler explanation that backcrossing is occurring and, as will be discussed, is biased toward one of the parents.

Fourteen (88%) of the individuals identified as backcrosses appeared to be introgressed toward *U. pumila* with only a two individuals backcrossing with *U. rubra*. Patterns of biased introgression are frequently observed in plants (Hedge et al., 2006) and have been reported in other tree species (Bacilieri et al., 1996; Keim et al., 1989). For example, in Europe, the sessile oak, *Quercus petraea*, has been progressively replacing the pedunculate oak, *Q. robur* (Bacilieri et al., 1996). In poplars, F_1 hybrids of *Populus fremontii* and *P. angustifolia* are commonly found together with backcrosses to *P. angustifolia*; however, backcrosses to *P. fremontii* are rarely found (Keim et al., 1989). Such asymmetrical patterns of introgression can occur when the parental species differ significantly in abundance (Ellstrand and Elam, 1993; Burgess et al., 2005) or when genetic incompatibilities occur (Keim et al., 1989). We did identify 32 individuals of *U. pumila* in the contact zones and only nine individuals of *U. rubra*. Moreover, *U. pumila* is DED tolerant, and thus trees of this species are less likely to be lost or weakened by the disease. In contrast, *U. rubra* is susceptible to DED, and its populations have been severely affected by the disease (Lester and Smalley, 1972a, b) with many populations consisting of only young or diseased trees that produce little or no pollen. However, pollen may travel long distances in this species, and thus the abundance of parental species in the contact zone may or not be a strong limiting factor to hybridization. The detection of some backcrosses with *U. rubra* does not support the genetic incompatibility mechanism. Whatever the mechanism responsible for the biased introgression, the recurrent backcrossing with *U. pumila* suggests that the majority of *U. rubra* genes will eventually be lost. Thus, the pattern of hybridization and introgression does not favor the maintenance of *U. rubra* genes in natural landscapes where *U. pumila* is present. Although in itself hybridization is unlikely to lead to the genetic swamping of *U. rubra*, a fairly widespread species, hybridization and biased introgression back to *U. pumila* can be added to forest fragmentation and DED as factors affecting the long-term conservation of *U. rubra* in the United States. Therefore, assessing the current level of genetic variation in uncontaminated *U. rubra* natural populations is important as an aide for the conservation of genetic resources (Cogolludo-Agustin et al., 2000; Goodall-Copestake et al., 2005).

The third goal of this study was to describe the genetic diversity in the parental populations and the hybrids and to determine whether the hybrids had more genetic diversity relative to either one of their parental species. The analysis of molecular variance indicated significant levels of genetic variation both within and between our parental species as well as the hybrid groups. We found similar numbers of alleles in the hybrids and in *U. rubra,* and fewer alleles in *U. pumila,* although different numbers of individuals were sampled in each group. When sample sizes were considered, we found an average of 7.4 alleles in 125 individuals in *U. rubra* and 7.2 alleles in 51 individuals in the hybrids, suggesting that the hybrids may capture more alleles overall, assuming that more alleles would be discovered in the hybrids if the sample size were larger. In addition, hybrid individuals have new combinations of alleles not observed in either of the parental species because they harbor alleles from both of the parental species. We observed an excess of heterozygotes in the hybrid group, which is expected given the presence of many F_1 individuals. Moreover, the Shannon index of diversity (I) and the level of heterozygosity were both highest in the hybrid group [HY(HY)]. While the two parental species were genetically well-differentiated (F_{ST} = 0.50), the levels of genetic differentiation between each parental species and the hybrids were similar, mostly due to the overabundance of F_1 hybrids. Given the common and widespread patterns of hybridization observed between *U. rubra* and *U. pumila* in Wisconsin and the capacity for other elm species to hybridize, the possibility exists that the invasiveness reported for *U. pumila* in various regions of the United States could be attributed to recent hybridization with one or more native elm species. It has been suggested that hybridization between two species can serve as a stimulus for the evolution of invasiveness by increasing genetic diversity and creating new genotypes (Ellstrand and Schierenbeck, 2000). Future studies will determine whether pure *U. pumila* populations exist in Wisconsin or whether populations consist mostly of hybrids between *U. pumila* and *U. rubra*. Moreover, given the weak incompatibility barriers in the genus *Ulmus* (Lester and Smalley, 1972a, b; Santamour, 1972; Townsend, 1975; Wiegrefe et al., 1994), future studies are needed to determine whether *U. pumila* naturally hybridize and introgress with other native elms in North America. The nine microsatellite loci described here, together with the unique fingerprint profiles for *U. rubra* and *U. pumila*, will facilitate studies of diversity and in-depth analysis of hybridization between these and other elm species.

Conclusions—Hybridization between *U. rubra* and *U. pumila* is extensive and introgression is biased back toward *U. pumila*. Such hybridization may have affected the potential for

invasiveness *U. pumila* and threatens the long-term survival of *U. rubra* in the United States. Hybridization could be advantageous for *U. rubra* due to transmission of DED resistance genes from *U. pumila* if the introgression was occurring toward this species. However, our results clearly show that introgression is preferential toward *U. pumila*; therefore, likely enhancing the fitness of *U. pumila* by acquiring useful genes from *U. rubra* and potentially leading to the extinction of the latter species. Also, it is notable that *U. rubra*, devastated by Dutch elm disease, still possess high genetic variation, even higher than the level of genetic variation in *U. pumila*. Additional studies will be required to assess diversity of *U. rubra* to confirm this pattern. We suggest that the long generation time in *Ulmus* may act as buffer and account for the relatively high genetic diversity still present in *U. rubra*.

LITERATURE CITED

Baack, E. J., and L. H. Rieseberg. 2007. A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics & Development* 17: 513–518.

Bacilieri, R., A. Ducousso, R. J. Petit, and A. Kremer. 1996. Mating system and asymmetric hybridization in a mixed stand of European oaks. *Evolution* 50: 900–908.

Brasier, C. M. 1988. *Ophiostoma ulmi*, cause of Dutch elm disease. *Advances in Plant Pathology* 6: 207–221.

Brasier, C. M. 1991. *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. *Mycopathologia* 115: 151–161.

Burgess, K. S., M. Morgan, L. Deverno, and B. C. Husband. 2005. Asymmetrical introgression between two *Morus* species (*M. alba, M. rubra*) that differ in abundance. *Molecular Ecology* 14: 3471–3483.

Cogolludo-Agustín, M. A., D. Agúndez, and L. Gil. 2000. Identification of native and hybrid elms in Spain using isozyme gene markers. *Heredity* 85: 157–166.

Collada, C., P. Fuentes-Utrilla, L. Gil, and M. T. Cervera. 2004. Characterization of microsatellite loci in *Ulmus minor* Miller and cross-amplification in *U. glabra* Hudson and *U. laevis* Pall. *Molecular Ecology Notes* 4: 731–732.

Collin, E. 2002. Strategies and guidelines for the conservation of the genetic resources of *Ulmus* spp. *In* Turok, J, G. Eriksson, K. Russell, and S. Borelli [eds.], Noble Hardwoods Network, 50–67, Report of the fourth meeting, September 1999, Gmunden, Austria, and the fifth meeting, May 2001, Blessington, Ireland. International Plant Genetic Resources Institute, Rome, Italy.

Daehler, C., and D. Strong. 1997. Hybridization between introduced smooth cordgrass (*Spartina alterniflora*; Poaceae) and native California cordgrass (*S. foliosa*) in San Francisco Bay, California, USA. *American Journal of Botany* 84: 607–611.

Ding, J., R. Reardon, Y. Wu, H. Zheng, and W. Fu. 2006. Biological control of invasive plants through collaboration between China and the United States of America: A perspective. *Biological Invasions* 8: 1439–1450.

Ellstrand, N. C., and D. Elam. 1993. Population genetic consequences of small population size: Implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217–242.

Ellstrand, N. C., and K. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences, USA* 97: 7043–7050.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.

Goodall-Copestake, W. P., M. L. Hollingsworth, P. M. Hollingsworth, G. I. Jenkins, and E. Collin. 2005. Molecular markers and *ex situ* conservation of the European elms (*Ulmus* spp.). *Biological Conservation* 122: 537–546.

Hedge, S. G., J. D. Nason, J. M. Clegg, and N. C. Ellstrand. 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. *Evolution* 60: 1187–1197.

Keim, P., K. N. Paige, T. G. Whitham, and K. G. Lark. 1989. Genetic analysis of an interspecific hybrid swarm of *Populus*: Occurrence of unidirectional introgression. *Genetics* 123: 557–565.

Lester, D. T., and E. B. Smalley. 1972a. Response of *Ulmus pumila* and *U. pumilia* × *rubra* hybrids to inoculation with *Ceratocystis ulmi. Phytopathology* 62: 848–852.

Lester, D. T., and E. B. Smalley. 1972b. Variation in ornamental traits and disease tolerance among crosses of *Ulmus pumila, U. rubra* and putative natural hybrids. *Silvae Genetica* 21: 193–197.

Londo, J. P., and B. A. Schaal. 2007. Origins and population genetics of weedy red rice in the USA. *Molecular Ecology* 16: 4523–4535.

Machon, N., M. Lefranc, I. Bilger, S. J. Mazer, and A. Sarr. 1997. Allozyme variation in *Ulmus* species from France: analysis of differentiation. *Heredity* 78: 12–20.

Mittempergher, L., and A. Santini. 2004. The history of elm breeding. *Investigación Agraria*, *Sistemas y Recursos Forestales* 13: 161–177.

Moody, M., and D. Les. 2007. Geographic distribution and genotypic composition of invasive hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*) populations in North America. *Biological Invasions* 9: 559–570.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Prentis, P. J., E. M. White, I. J. Radford, A. J. Lowe, and A. R. Clarke. 2007. Can hybridization cause local extinction: A case for demographic swamping of the Australian native S*enecio pinnatifolius* by the invasive *Senecio madagascariensis? New Phytologist* 176: 902–912.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* 27: 83–109.

Rieseberg, L. H., S. Zona, L. Aberbom, and T. D. Martin. 1989. Hybridization in the island endemic, Catalina mahogany. *Conservation Biology* 3: 52–58.

Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J. L. Durphy, et al. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301: 1211–1216.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, et al. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305–332.

Santamour, F. S. 1972. Interspecific hybridization with fall- and spring-flowering elms. *Forest Science* 18: 283–289.

Santini, A., N. La Porta, L. Ghelardini, and L. Mittempergher. 2008. Breeding against Dutch elm disease adapted to the Mediterranean climate. *Euphytica* 163: 45–56.

Smalley, E. B., and R. P. Guries. 1993. Breeding elms for tolerance to Dutch elm disease. *Annual Review of Phytopathology* 31: 325–352.

Smouse, P. E., and R. Peakall. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* 82: 561–573.

Strauss, S. Y., J. A. Lau, and S. P. Carroll. 2006. Evolutionary responses of natives to introduced species: What do introductions tell us about natural communities? *Ecology Letters* 9: 357–374.

Townsend, A. M. 1975. Crossability patterns and morphological variation among elm species and hybrids. *Silvae Genetica* 24: 18–23.

USDA, NRCS. 2002. PLANTS Database, version 3.5 [online, website http://plants.usda.gov]. National Plant Data Center, Baton Rouge, Louisiana, USA.

Vila, M., E. Weber, and C. M. D'Antonio. 2000. Conservation implications of invasion by plant hybridization. *Biological Invasions* 2: 207–217.

Ware, G. H. 1995. Little-known elms from China: Landscape tree possibilities. *Journal of Arboriculture* 21: 284–288.

Whiteley, R. E., S. Black-Samuelsson, and D. Clapham. 2003. Development of microsatellite markers for the European

white elm (*Ulmus laevis* Pall.) and cross-species amplification within the genus *Ulmus*. *Molecular Ecology Notes* 3: 598–600.

Wiegrefe, S. J., K. J. Sytsma, and R. P. Guries. 1994. Phylogeny of elms (*Ulmus*, Ulmaceae): Molecular evidence for a sectional classification. *Systematic Botany* 19: 590–612.

Wolfe, L., A. Blair, and B. Penna. 2007. Does intraspecific hybridization contribute to the evolution of invasiveness?: An experimental test. *Biological Invasions* 9: 515–521.

Wyman, D. 1951. Elms grown in America. *Arnoldia* 11: 79–93.

Zalapa, J. E., J. Brunet, and R. P. Guries. 2008a. Isolation and characterization of microsatellite markers for red elm (*Ulmus rubra* Muhl.) and cross-species amplification with Siberian elm (*Ulmus pumila* L.). *Molecular Ecology Resources* 8: 109–112.

Zalapa, J. E., J. Brunet, and R. P. Guries. 2008b. Genetic diversity and relationships among Dutch elm disease-tolerant *Ulmus pumila* L. accessions from China. *Genome* 51: 492–500.

Appendix 1. *Ulmus rubra* herbarium specimens used in the study of hybridization between *U. rubra* and *U. pumila*. Samples collected from the dry collection housed at the University of Wisconsin-Madison Herbarium.

Accession	Date	County
v0166417WIS	6/24/1958	Brown
v0166418WIS	8/23/1948	Calumet
v0166419WIS	5/26/1951	Clark
v0166424WIS	9/3/1932	Crawford
v0166430WIS	5/18/1965	Dane
v0166433WIS	5/11/1965	Dane
v0166435WIS	5/6/1957	Dane
v0166698WIS	7/22/1956	Grant
v0166712WIS	5/12/1940	Iowa
v0166716WIS	5/21/1959	Iowa
v0166719WIS	5/11/1960	Iowa
v0166723WIS	5/20/1936	Jefferson
v0166726WIS	8/29/1956	La Crosse
v0166728WIS	7/9/1957	Lafayette
v0166733WIS	5/18/1952	Lincoln
v0166734WIS	7/28/1952	Lincoln
v0166735WIS	5/18/1952	Lincoln
v0166737WIS	8/14/1950	Lincoln
v0166738WIS	6/5/1951	Lincoln
v0166742WIS	4/25/1890	Outagamie
v0167091WIS	5/26/1960	Sauk
v0167092WIS	5/19/1960	Sauk
v0167098WIS	7/13/1922	Vernon
v0167103WIS	6/14/1947	Waukesha
v0167104WIS	9/26/1938	Waukesha

APPENDIX 2. Genetic profile and diversity characteristics for *Ulmus rubra*, *U. pumila*, and putative hybrids based on species-specific allelic variation at nine microsatellite loci. Allele frequencies, N = number of individuals; A_o = observed number of alleles, A_e = effective number of alleles; I = Shannon index of diversity; H_o = observed heterozygosity; and H_e = expected heterozygosity. Bold alleles = *U. rubra* alleles; plain = *U. pumila* alleles; and italics = unique alleles in hybrids (only in marker UR188).

		Parental and putative hybrids			Hybrid identification			Hybrid class	
Locus	Allele	RU	PU	HY	RU(HY)	PU(HY)	HY(HY)	F_1(HY)	B_C(HY)
UR123	239	**—**	0.05	0.01	—	0.02	—	—	—
	241	**—**	0.06	0.01	—	0.02	—	—	—
	243	**—**	0.10	0.05	—	0.06	0.04	0.03	0.07
	244	**0.004** [b]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
	245	**—**	0.16	0.20	—	0.27	0.20	0.23	0.14
	246	**0.07** [a,b]	**—**	**0.01**	**—**	**—**	**0.02**	**0.02**	**0.04**
	248	**0.11** [a,b]	**—**	**0.05**	**0.17**	**—**	**0.06**	**0.06**	**0.07**
	251	**0.08** [a,b]	**—**	**0.01**	**—**	**—**	**0.02**	**0.03**	**—**
	252	**0.13** [a,b]	**—**	**0.04**	**0.11**	**—**	**0.05**	**0.06**	**0.04**
	253	—	0.47	0.29	—	0.50	0.20	0.18	0.25
	254	**0.53** [a,b]	**—**	**0.22**	**0.72**	**—**	**0.28**	**0.29**	**0.25**
	255	—	0.09	0.06	—	0.06	0.06	0.06	0.07
	256	**0.01** [a]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
	257	—	0.01	—	—	—	—	—	—
	258	**0.004** [a]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
	259	—	0.03	0.01	—	—	0.01	—	0.04
	260	**0.04** [a,b]	**—**	**0.01**	**—**	**—**	**0.01**	**0.02**	**—**
	261	—	0.01	0.01	—	0.02	—	—	—
	262	**0.01** [a,b]	**—**	**0.01**	**—**	**—**	**0.02**	**0.02**	**0.04**
	263	—	0.02	0.02	—	0.06	—	—	—
	264	**0.01** [a]	**—**	**0.01**	**—**	**—**	**0.01**	**0.02**	**—**
	268	**0.004** [a]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
N		115	50	88	9	32	47	33	14
A_o		12	10	17	3	8	13	12	10
A_e		3.15	3.67	5.39	1.78	3	5.79	5.51	6.03
I		1.59	1.69	2.02	0.78	1.41	2.03	1.98	2.01
H_o		0.70	0.76	0.80	0.56	0.63	0.96	1	0.86
H_e		0.68	0.73	0.81	0.44	0.67	0.83	0.82	0.83
UR141	150	—	1	0.66	—	1	0.56	0.50	0.71
	152	**0.01** [a,b]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
	154	**0.99** [a,b]	**—**	**0.34**	**1**	**—**	**0.44**	**0.50**	**0.29**
N		124	51	92	9	32	51	35	16
A_o		2	1	2	1	1	2	2	2
A_e		1.02	1	1.81	1	1	1.97	2	1.69
I		0.05	—	0.64	—	—	0.69	0.69	0.60
H_o		0.02	—	0.45	—	—	0.83	1	0.43
H_e		0.02	—	0.45	—	—	0.49	0.50	0.41
UR153	178	—	0.48	0.28	—	0.38	0.26	0.24	0.32
	184	**0.004** [a]	**—**	**0.01**	**—**	**—**	**0.01**	**0.01**	**—**
	186	**0.03** [a,b]	**—**	**0.01**	**0.11**	**—**	**—**	**—**	**—**
	187	—	0.09	0.16	—	0.31	0.09	0.10	0.07
	189	**0.61** [a,b]	**—**	**0.11**	**0.06**	**—**	**0.20**	**0.21**	**0.18**
	191	—	0.01	0.01	—	0.02	—	—	—
	193	**0.07** [a,b]	**—**	**0.04**	**0.22**	**—**	**0.03**	**0.03**	**0.04**
	194	**0.02** [a,b]	**—**	**0.02**	**—**	**—**	**0.03**	**0.03**	**0.04**
	196	**0.25** [a,b]	**—**	**0.13**	**0.33**	**—**	**0.18**	**0.21**	**0.11**
	197	—	0.38	0.21	—	0.30	0.19	0.16	0.25
	199	**0.01** [a]	**—**	**0.03**	**0.28**	**—**	**0.01**	**0.01**	**—**

APPENDIX 2. Continued.

Locus	Allele	Parental and putative hybrids			Hybrid identification			Hybrid class	
		RU	PU	HY	RU(HY)	PU(HY)	HY(HY)	F_1(HY)	B_C(HY)
	200	—	0.03	—	—	—	—	—	—
	208	—	0.01	—	—	—	—	—	—
N		115	49	91	9	32	50	35	15
A_o		7	6	11	5	4	9	9	7
A_e		2.25	2.62	5.62	3.95	3.06	5.42	5.58	4.61
I		1.10	1.14	1.91	1.46	1.16	1.83	1.85	1.68
H_o		0.54	0.49	0.81	0.78	0.63	0.94	1	0.79
H_e		0.56	0.62	0.82	0.75	0.67	0.82	0.82	0.78
UR158	180	—	0.95	0.63	—	0.97	0.52	0.50	0.57
	184	**0.01** [a,b]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
	187	—	0.05	0.01	—	0.03	—	—	—
	196	**0.04** [a,b]	**—**	**0.01**	**—**	**—**	**0.02**	**0.01**	**0.04**
	200	**0.83** [a,b]	**—**	**0.31**	**0.89**	**—**	**0.41**	**0.44**	**0.32**
	205	**0.13** [a,b]	**—**	**0.04**	**0.11**	**—**	**0.05**	**0.04**	**0.07**
N		124	49	91	9	32	50	35	15
A_o		4	2	5	2	2	4	4	4
A_e		1.43	1.11	2.03	1.25	1.06	2.28	2.24	2.29
I		0.58	0.20	0.88	0.35	0.14	0.94	0.91	0.99
H_o		0.27	0.10	0.53	0.22	0.06	0.90	1	0.64
H_e		0.30	0.10	0.51	0.20	0.06	0.56	0.55	0.56
UR159	**236**	**0.05** [a,b]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
	245	**0.95** [a,b]	**—**	**0.35**	**1**	**—**	**0.47**	**0.50**	**0.39**
	249	—	0.03	0.09	—	0.11	0.09	0.09	0.11
	257	—	0.96	0.56	—	0.89	0.44	0.41	0.50
	279	—	0.01	—	—	—	—	—	—
N		120	51	91	9	32	50	34	16
A_o		2	3	3	1	2	3	3	3
A_e		1.10	1.08	2.26	1	1.24	2.38	2.34	2.40
I		0.19	0.19	0.91	—	0.35	0.94	0.93	0.95
H_o		0.09	0.08	0.58	—	0.22	0.94	1	0.79
H_e		0.09	0.08	0.56	—	0.19	0.58	0.57	0.58
UR173a	137	—	0.06	0.01	—	—	0.02	0.02	0.04
	146	—	0.94	0.61	—	1	0.46	0.48	0.54
	156	**0.02** [a,b]	**—**	**0.01**	**—**	**—**	**0.01**	**0.02**	**—**
	158	**0.07** [a,b]	**—**	**0.02**	**0.06**	**—**	**0.02**	**0.03**	**—**
	160	**0.18** [a,b]	**—**	**0.17**	**0.44**	**—**	**0.24**	**0.15**	**0.21**
	162	**0.14** [a,b]	**—**	**0.03**	**—**	**—**	**0.05**	**0.07**	**0.04**
	164	**0.19** [a,b]	**—**	**0.06**	**0.22**	**—**	**0.06**	**0.03**	**0.14**
	166	**0.11** [a,b]	**—**	**0.02**	**—**	**—**	**0.03**	**0.05**	**—**
	168	**0.06** [a,b]	**—**	**0.04**	**0.11**	**—**	**0.05**	**0.08**	**—**
	170	**0.04** [a,b]	**—**	**0.01**	**—**	**—**	**0.01**	**0.02**	**—**
	172	**0.07** [a,b]	**—**	**0.03**	**0.17**	**—**	**0.02**	**0.03**	**—**
	174	**0.06** [a,b]	**—**	**0.01**	**—**	**—**	**0.02**	**0.02**	**0.04**
	177	**0.01** [a]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
	178	**0.02** [a]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
	180	**0.02** [a,b]	**—**	**—**	**—**	**—**	**—**	**—**	**—**
N		122	49	91	9	32	50	35	15
A_o		13	2	12	5	1	12	12	6
A_e		8.17	1.13	2.47	3.45	1	3.57	3.64	2.80
I		2.28	0.23	1.39	1.40	—	1.71	1.79	1.30
H_o		0.93	0.04	0.53	0.89	—	0.81	1	0.64
H_e		0.88	0.11	0.59	0.71	—	0.72	0.73	0.64

APPENDIX 2. Continued.

Locus	Allele	Parental and putative hybrids			Hybrid identification			Hybrid class	
		RU	PU	HY	RU(HY)	PU(HY)	HY(HY)	F_1(HY)	B_C(HY)
UR188a	108	—	1	0.65	—	1	0.53	0.50	0.61
	112	**0.004** [a]	—	—	—	—	—	—	—
	114	**0.06** [a,b]	—	**0.04**	**0.22**	—	**0.03**	**0.03**	**0.04**
	116	**0.004** [b]	—	—	—	—	—	—	—
	120	**0.08** [a,b]	—	**0.01**	—	—	**0.01**	**0.01**	—
	122	**0.26** [a,b]	—	**0.11**	**0.33**	—	**0.14**	**0.18**	**0.04**
	124	**0.39** [a,b]	—	**0.12**	**0.28**	—	**0.17**	**0.15**	**0.21**
	125	**0.07** [a,b]	—	**0.02**	—	—	**0.03**	**0.04**	—
	127	**0.04** [a,b]	—	**0.01**	—	—	**0.01**	**0.01**	—
	129	**0.03** [a]	—	—	—	—	—	—	—
	131	**0.03** [a]	—	**0.01**	**0.11**	—	—	—	—
	132	**0.02** [a,b]	—	—	—	—	—	—	—
	134	**0.01** [a]	—	**0.01**	—	—	**0.02**	**0.01**	**0.04**
	136	**0.01** [a,b]	—	—	—	—	—	—	—
	138	—	—	*0.01*	—	—	*0.01*	—	*0.04*
	140	—	—	*0.03*	*0.06*	—	*0.05*	*0.06*	*0.04*
	142	**0.004** [a]	—	—	—	—	—	—	—
	144	**0.004** [b]	—	—	—	—	—	—	—
N		122	51	92	9	32	51	35	16
A_o		15	1	11	5	1	10	9	7
A_e		4.28	1	2.24	3.95	1	3	3.23	2.38
I		1.85	—	1.27	1.46	—	1.50	1.53	1.23
H_o		0.84	—	0.57	0.78	—	0.92	1	0.71
H_e		0.77	—	0.55	0.75	—	0.67	0.69	0.58
ULMI98	122	—	0.25	0.06	—	0.11	0.04	0.03	0.07
	124	—	0.32	0.21	—	0.25	0.22	0.21	0.25
	126	**0.04** [a,b]	—	—	—	—	—	—	—
	128	—	0.19	0.15	—	0.28	0.09	0.10	0.07
	130	—	0.08	0.05	—	0.06	0.05	0.06	0.04
	134	—	0.14	0.17	—	0.30	0.13	0.10	0.18
	136	—	0.03	—	—	—	—	—	—
	143	**0.04** [a,b]	—	**0.03**	**0.22**	—	**0.01**	**0.01**	—
	145	**0.53** [a,b]	—	**0.26**	**0.50**	—	**0.39**	**0.41**	**0.32**
	146	**0.01** [a]	—	—	—	—	—	—	—
	147	**0.28** [a,b]	—	**0.04**	**0.11**	—	**0.06**	**0.06**	**0.07**
	148	**0.06** [a,b]	—	**0.01**	—	—	**0.01**	**0.01**	—
	150	**0.004** [a]	—	—	—	—	—	—	—
	151	**0.02** [a,b]	—	—	—	—	—	—	—
	152	**0.004** [a]	—	**0.01**	**0.06**	—	—	—	—
	165	**0.004** [a]	—	**0.01**	**0.11**	—	—	—	—
N		123	51	89	9	32	48	34	14
A_o		10	6	11	5	5	9	9	7
A_e		2.69	4.44	5.79	3.06	4.07	4.36	4.14	4.67
I		1.31	1.60	1.94	1.33	1.48	1.74	1.72	1.70
H_o		0.52	0.78	0.88	0.67	0.84	0.94	1	0.79
H_e		0.63	0.77	0.83	0.67	0.75	0.77	0.76	0.79
ULM2	100	—	1	0.64	—	1	0.52	0.50	0.57
	103	**0.56** [a,b]	—	**0.18**	**0.50**	—	**0.24**	**0.25**	**0.21**
	106	**0.44** [a,b]	—	**0.18**	**0.50**	—	**0.24**	**0.25**	**0.21**
N		123	51	92	9	32	51	35	16
A_o		2	1	3	2	1	3	3	3

APPENDIX 2. Continued.

Locus	Allele	Parental and putative hybrids			Hybrid identification			Hybrid class	
		RU	PU	HY	RU(HY)	PU(HY)	HY(HY)	F_1(HY)	B_C(HY)
A_e		1.97	1	2.11	2	1	2.59	2.67	2.39
I		0.69	—	0.90	0.69	—	1.02	1.04	0.98
H_o		0.54	—	0.55	0.78	—	0.88	1	0.57
H_e		0.49	—	0.53	0.50	—	0.61	0.63	0.58

Notes: RU = Wisconsin U. rubra population + U. rubra herbarium; PU = Chinese U. pumila individuals; HY = all individuals from putative hybrid populations; RU(HY) = U. rubra individuals from contact zones; PU(HY) = U. pumila individuals from contact zones; HY(HY) = all confirmed hybrid individuals from contact zones; F_1(HY) = confirmed first-generation hybrids; B_C(HY) = confirmed backcrossed hybrids.

[a] *U. rubra* populations alleles;

[b] *U. rubra* herbarium alleles