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Molecular evidence for a single genetic clone of invasive *Arundo donax* in the United States

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Abstract

Arundo donax (giant reed) is an aggressive invasive weed of riparian habitats throughout the southern half of the United States from California to Maryland. Native to Asia, the species is believed to have been initially introduced into North America from the Mediterranean region although subsequent introductions were from multiple regions. To provide insight into the potential for biological control of *A. donax*, genetic variation in plants sampled from a wide geographical area in the United States was analyzed using Sequence Related Amplification Polymorphism (SRAP) and transposable element (TE)-based molecular markers. Invasive individuals from 15 states as well as four populations in southern France were genetically fingerprinted using 10 SRAP and 12 TE-based primer combinations. With the exception of simple mutations detected in four plants, *A. donax* exhibited a single multilocus DNA fingerprint indicating a single genetic clone. The genetic uniformity of invasive *A. donax* suggests that classical biological control of the species could be successful. A lack of genetic diversity in the invaded range simplifies identification of native source populations to search for natural enemies that could be used as biocontrol agents.
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1. Introduction

Arundo donax L. is a tall, perennial reed-like grass found in riparian habitats throughout the southern half of the United States from California to Maryland (Bell, 1994, 1997; DiTomaso and Healey, 2003; Dudley, 2000). Believed to be native to freshwaters of eastern Asia (Polunin and Huxley, 1987), *A. donax* has been cultivated throughout Asia, southern Europe, north Africa, and the Middle East for thousands of years (Perdue, 1958; Zohary, 1962) and has naturalized in the countries surrounding the Mediterranean Sea (Lewandowski et al., 2003). From this area, the species was widely dispersed into subtropical and warm temperate regions around the world by humans for multiple uses (Perdue, 1958). Within North America, *A. donax* is believed to have been initially introduced into southern California from the Mediterranean in the early 1800s for erosion control. It then also came to be used for roof thatching and cultivated for the production of reeds for musical instruments (Bell, 1997; Hoshovsky, 1987). Since its introduction into North America, *A. donax* has escaped cultivation and become a major invasive weed of riparian habitats where it not only displaces native species but also dramatically modifies ecological and successional processes (Bell, 1994, 1997; DiTomaso and Healey, 2003; Dudley, 2000).

The success of *A. donax* as an invasive species in riparian areas is at least partly attributable to its rapid clonal spread by rhizome extension and flood dispersal of rhizome and stem fragments (DiTomaso and Healey, 2003; Dudley, 2000), which makes physical removal problematic. Although *A. donax* produces flowers, viable seed has not been observed in most areas where it has been introduced (Perdue, 1958), including North America (DiTomaso and Healey, 2003; Dudley, 2000). The apparent lack of sexual reproduction, and low genetic diversity expected, are viewed positively when evaluating *A. donax* for its suitability for classical biological control (Tracy and DeLoach, 1999). In general, asexual weeds have been observed to be more effectively controlled by biological agents than sexually reproducing weeds (Burdon and Marshall, 1981), presumably because a lack of adaptive genetic variation in asexual populations limits the capacity to evolve resistance to herbivore and pathogen attack (Müller-Schärer and Steinger, 2004).

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In the last decade, multilocus molecular markers capable of genotyping or DNA fingerprinting individuals with high resolution have been widely used to characterize genetic variation in invasive plants (e.g. Genton et al., 2005; Saltonstall, 2003; Williams et al., 2005). In general, however, variation at selectively neutral marker loci does not reflect a population's capacity for adaptive evolutionary change to biological control agents (Müller-Schärer and Steinger, 2004; Reed and Frankham, 2001). Rather, adaptive evolution depends on genetic variation in fitness-related traits, such as growth rate, phenology, and resistance to herbivores (Lande, 1988). The degree and structuring of neutral genetic variation can, however, indicate the number of introductions, geographic and genetic origins, and pathways of spread of invasive genotypes in invaded ranges. Such information is critical for identifying areas to search for natural enemies that could be used as biological control agents (Roderick, 2004; Roderick and Navajas, 2003) and thus can provide insight into the suitability of classical biological control for an invasive weed.

In this study, we investigate genetic variation in the invasive species *A. donax* across the invaded range of the United States. In an earlier study, Khudamrongsawat et al. (2004) studied genetic diversity, using isozyme and Randomly Amplified Polymorphic DNA (RAPD) markers, in *A. donax* along the Santa Ana River in California and concluded that biological control could be successful. The genetic diversity of invasive *A. donax* at a wider geographical scale is not known, however. The specific objective of this study was to investigate genetic variation in *A. donax* across a wide geographic area in the United States. Our results will contribute to the planning of research directed toward the biological control of this highly invasive species of riparian habitats.

2. Materials and methods

2.1. Plant sampling

We sampled a total of 185 putative clones of invasive *A. donax* across the United States (Fig. 1, Table 1). Sampling locations were determined by contacting state invasive plant councils, managers of wildlife preserves and conservation areas, university and government researchers, and state and university weed extension personnel. In addition, we conducted Internet searches and examined herbarium material for locations of invasive *A. donax*. Once an extensive list of locations was identified, we mapped out a sampling route that utilized the interstate highway system for long distance travel and local roads for travel to specific sites. If the opportunity presented itself, we also spoke with local residents who sometimes identified additional sampling locations.

At each sampling location, we obtained young leaf tissue from putative clones, i.e. discrete clumps or clusters of stems (*sensu* Spencer et al., 2006) that were a minimum of 5 m apart from one another. Often, leaf samples were obtained from multiple stems of a single *A. donax* clump or cluster. In such instances, leaf samples from each stem of a cluster were stored and DNA fingerprinted independently. We also obtained leaf material from 20 randomly selected putative clones of invasive *A. donax* within four populations in southern France (Table 1). Global Positioning System (GPS) coordinates were obtained for each invasive clone sampled and are available upon request from the authors. All leaf tissue sampled was stored in plastic zipper bags containing silica gel for shipping or transport (Chase and Hills, 1991) to the laboratory then immediately frozen and stored at -80 °C until DNA extraction.

Genetic diversity in *A. donax* was expected to be low based on previous reports (e.g. Khudamrongsawat et al., 2004; Lewandowski et al., 2003) and the observed lack of seed production in North America (DiTomaso and Healey, 2003; Dudley, 2000). Thus, we also obtained leaf material from six ornamental plantings and three cultivars ('Golden Chain', 'Macrophylla', and 'Variegata') of *A. donax*, and two additional cultivated clones sold as *A. formosana* ('Oriental Gold') and *A. nobilis* ('Variegata') to confirm the polymorphism of molecular markers used to fingerprint invasive *A. donax*. Cultivars of *A. donax*, *A. formosana*, and *A. nobilis* were obtained from nurseries and botanical gardens whereas *A. donax* ornamental plantings were sampled from residential landscapes during the

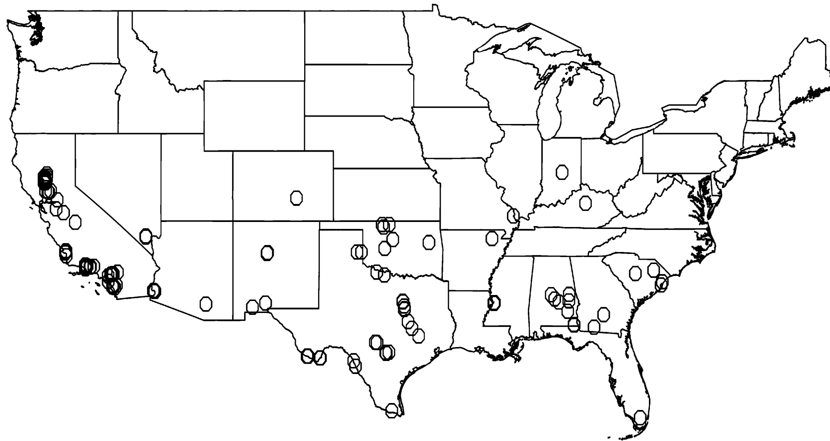

Fig. 1. Map of the continental United States showing the geographical distribution of *Arundo donax* sampled for this study. Each open circle represents the location of a clump or cluster (putative clone) of stems sampled. Multiple circles in a specific area indicate samples were collected from multiple clumps in the area. GPS coordinates of sampling locations are available upon request from the authors.

Table 1
Summary of sampling regions, and numbers of putative clones[a] and leaves sampled and genetically fingerprinted in each region

Species	Sampling region	Number of putative clones sampled	Number of leaf samples DNA fingerprinted
Invasive *A. donax*	United States		
	Alabama	8	23
	Arizona	4	4
	Arkansas	1	2
	California	95 (1)[b]	121 (1)[c]
	Colorado	1	2
	Florida	4	4
	Georgia	2 (1)	6 (3)
	Kentucky	1	3
	Louisiana	1 (1)	3 (3)
	Mississippi	8	8
	Nevada	2	2
	New Mexico	10	21
	Oklahoma	12 (4)	14 (4)
	South Carolina	6	6
	Texas	30 (1)	71 (3)
	Southern France		
	Caund' Arago	5	5
	St. Jean de Cuculles	5	5
	Castelnau le Lez	5	5
	Montferrier sur Lez	5	5
Cultivated *Arundo*			
A. donax ornamental plantings	US: Florida	2 (1)	2 (1)
	US: Indiana	1	1
	US: Kentucky	1 (1)	3 (3)
	US: Missouri	1	3
	UK: Scotland	1	1
A. donax 'Golden Chain'	US: North Carolina	1	1
A. donax 'Macrophylla'	UK: England	1	1
A. donax 'Variegata'	US: North Carolina	1 (1)	1 (1)
A. donax 'Variegata'	UK: Scotland	1 (1)	1 (1)
A. formosana 'Oriental Gold'	US: California	1	1
A. nobilis 'Variegata'	US: Oregon	1 (1)	1 (1)

[a] A putative clone is a discrete clump or cluster of stems that is likely to be a clone.
[b] Number in parenthesis indicates the number of putative clones of the total with variegated leaves.
[c] Number in parenthesis indicates the number of leaves of the total that were variegated.

road trip conducted to sample invasive *A. donax*. Ornamental plantings could not be identified to specific cultivars. All cultivated leaf samples were stored as described above for shipping or transport to the laboratory then immediately frozen and stored at −80 °C until DNA extraction.

2.2. DNA extraction

Genomic DNA was extracted from 200 to 300 mg of frozen leaf tissue using the CTAB procedure of Doyle and Doyle (1987) with addition of 10 μL of Proteinase K (20 mg mL^{-1}) to each sample after addition of CTAB buffer. DNA quality and quantity were determined visually from band intensities following standard horizontal electrophoresis on 1.0% agarose gels and ethidium bromide staining.

2.3. Molecular marker analyses

To increase the likelihood of detecting molecular marker variation that differentiates clones of *A. donax* and thus could potentially be used to identify geographic origins and locations to search for biological control agents, we employed molecular markers with higher resolution and wider genome coverage than those used by Khudamrongsawat et al. (2004). We used two classes of markers in this study: (1) Sequence Related Amplification Polymorphism (SRAP) markers and (2) Transposable Element (TE)-based markers.

2.3.1. SRAP markers

SRAP (Sequence Related Amplification Polymorphism) markers and the SRAP-PCR protocol (Li and Quiros, 2001) have been widely used to analyze intraspecific variation and differentiate closely related cultivars, inbred lines, and individuals of many plant species (e.g. Ahmad et al., 2004; Budak et al., 2004; Ferriol et al., 2003). Originally, the SRAP technique was developed to preferentially amplify open reading frames (ORFs) using combinations of two different primers each 17 or 18 nucleotides long with AT- and CG-rich core sequences (Li and Quiros, 2001). However, the specific primer design described in the original study is not necessary for

amplification. Rather, the unique feature of the SRAP procedure is the polymerase chain reaction (PCR) protocol, which can be used with primers of varying sequence, combination, and genome coverage. SRAP-PCR is run at the low annealing temperature of 35 °C for the first five cycles resulting in increased primer binding across the genome. Thereafter, the annealing temperature is increased to 50 °C for the last 35 cycles during which primers bind more specifically to regions of amplification products generated during the first 5 cycles of PCR. The SRAP-PCR method can be used with a variety of primer sequences and combinations, generates multiple fragments in a single PCR reaction, and does not require prior species-specific sequence information.

In this study, we used the SRAP-PCR protocol to initially evaluate 20 primer combinations for amplification, number of bands, and repeatability of results using DNA from four randomly selected *A. donax* clones and the *A. formosana* and *A. nobilis* clones. From the primers tested, 15 primer sequences (Table 2) and 10 primer combinations (Table 3) that generated the largest number of bands, and greatest repeatability of results and polymorphism were selected for analysis of all *Arundo* samples in this study. Using these primer combinations, DNA amplifications were carried out in a total reaction volume of 12 μL containing 20 ng of template DNA, 1× PCR buffer (QIAGEN, Valencia, CA) containing 1.5 mM $MgCl_2$, 208 nM of each primer, 200 μM of (each) dNTP, 1 unit of *Taq* polymerase (QIAGEN). SRAP-PCRs were performed following Li and Quiros (2001) in an MJ Research PTC-200 thermal cycler (Bio-Rad Laboratories, Inc., South San Francisco, CA) whereby the first five cycles were run at 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 1 min for denaturing, annealing and extension, respectively. An additional 35 cycles were run at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min. To denature reaction products for size separation, one μL of reaction product was mixed with 10 μL of formamide and 0.3 μL of CST ROX 50–650 Size Standard (Bioventures, Inc., Murfreesboro, TN) and held at 94 °C for 3 min then rapidly cooled on ice for 5 min.

2.3.2. TE-based markers

Transposable elements (TEs) have unique characteristics, including high copy number, dispersion throughout the genome, and in many cases, higher rates of evolution than conventional nuclear loci, that make them suitable molecular markers for differentiating closely related species and subspecies (Purugganan and Wessler, 1995). In addition, because TE activity is often restricted to specific strains or clones, TE-based molecular markers have the potential to distinguish reproductively isolated lines (Baumel et al., 2002), such as asexual clones, and thus be useful for detecting origins

Table 2
Primer sequences of SRAP and TE-based molecular markers used to genetically fingerprint *Arundo donax* in this study

Primer	Primer sequence	Source of sequence
SRAP markers		
ME1	TGAGTACAAACCGGAGA	Li and Quiros (2001)
ME2	TGAGTACAAACCGGAGC	Li and Quiros (2001)
ME3	TGAGTACAAACCGGAAT	Li and Quiros (2001)
MO1	GCAGACATGGCGACAAT	Ahmad et al. (2004)
TE1	TGTGTGGTTAATATGAGC	Ahmad et al. (2004)
E11	GACTGCGTACCAATTCAA	Vos et al. (1995)
E14	GACTGCGTACCAATTCAT	Vos et al. (1995)
E15	GACTGCGTACCAATTCCA	Vos et al. (1995)
E32	GACTGCGTACCAATTCAAC	Vos et al. (1995)
E39	GACTGCGTACCAATTCAGA	Vos et al. (1995)
E48	GACTGCGTACCAATTCAC	Vos et al. (1995)
E50	GACTGCGTACCAATTCCAT	Vos et al. (1995)
EM12	GACTGCGTACGAATTCTC	Modified Vos et al. (1995)
EM16	GACTGCGTACGAATTGTC	Modified Vos et al. (1995)
EM32	GACTGCGTACCAATTAAC	Modified Vos et al. (1995)
TE-based markers		
IRP-F	CTCGCTCGCCCACTACATCAACCGCGTTTATT	Kalendar et al. (1999)
IRP-R	CTGGTTCGGCCCATGTCTATGTATCCACACATGGTA	Kalendar et al. (1999)
RMP-R	GGAATTCATAGCATGGATAATAAACGATTATC	Kalendar et al. (1999)
BARE1	CTAGGGCATAATTCCAACAA	Gribbon et al. (1999)
TNA3	AGCCGTACTGAGATTCAC	Royo et al. (1996)
TNA6	ATTGTACTGCATACGTGG	Royo et al. (1996)
POK2	TTAACCCTTTTGACTAA	Penton et al. (2002)
M8387	CACACACACACACACACAG	Kalendar et al. (1999)
MF8081	GAGAGAGAGAGAGAGAGAC	Kalendar et al. (1999)
TEM1	AGTATTTTAGGAACGGAGGGAG	Chang et al. (2001)
TEM10	TCCCCACTTAGTGACCACGTCC	Chang et al. (2001)
POTT5	TTAGAAACACTCATTGGCCG	Oosumi and Belknap (1997)
ZMET21	CCAGCTCAGCTCAGATCTGTCATCCTTT	Papa et al. (2001)
STR187	AGACTAAGAGTCCTTTAAC	Berenyi et al. (2002)

Table 3
Primer combinations and number of amplification products (peaks) generated and shared between *Arundo* plants

Primer combination	Total no. amplification products (peaks)/No. shared with *Ad* 1[a]					
	Ad 1	*Ad* 2	*Ad* 3	*Ad* 4	*Af* 1	*An* 1
SRAP markers						
ME1 + E48	5/5	5/5	5/5	5/5	**7/3***	**6/2***
ME1 + EM16	13/13	13/13	13/13	13/13	**4/3***	**11/5***
ME2 + E11	23/23	23/23	23/23	23/23	**17/3***	**16/3***
ME2 + E14	16/16	16/16	16/16	16/16	**15/5***	**7/3***
ME2 + E39	15/15	**15/14***	15/15	15/15	**6/1***	**10/3***
ME3 + EM16	16/16	16/16	16/16	16/16	**16/7***	**11/4***
MO1 + TE 1	27/27	27/27	27/27	27/27	**22/7***	**22/5***
TE1 + E11	13/13	13/13	13/13	13/13	**7/3***	**6/3***
E15 + E32	40/40	40/40	40/40	40/40	**30/12***	**23/11***
EM32 + EM12	17/17	17/17	17/17	17/17	**13/5***	**18/6***
TE-based markers						
IRP-F + BARE1	17/17	17/17	17/17	17/17	**21/5***	**22/2***
IRP-F + TNA6	20/20	20/20	20/20	20/20	**18/5***	**21/7***
IRP-R + BARE1	18/18	18/18	18/18	18/18	**19/7***	**13/2***
IRP-R + TNA3	8/8	8/8	8/8	8/8	**8/1***	**4/0***
IRP-R + POK2	4/4	4/4	4/4	4/4	**7/4***	**5/3***
IRP-R + M8387	14/14	14/14	14/14	14/14	**10/3***	**6/1***
RMP-R + MF8081	9/9	9/9	**9/8***	**9/8***	**7/3***	**9/5***
TEM10 + POTT5	17/17	17/17	17/17	17/17	**12/7***	**20/8***
TEM10 + ZMET21	12/12	12/12	12/12	12/12	**5/4***	**14/9***
TEM1 + E48	27/27	27/27	27/27	27/27	**22/8***	**28/7***
STR187 + E50	6/6	6/6	6/6	6/6	**8/3***	**10/4***
Tn5ME1 + E39	12/12	12/12	12/12	12/12	**8/3***	**4/2***
Total no. clones	211	1	1	2	1	1

[a] Six unique DNA profiles (*Ad* 1, *Ad* 2, *Ad* 3, *Ad* 4, *Af* 1, and *An* 1) were detected in the samples fingerprinted where *Ad*: *Arundo donax*, *Af*: *A. formosana*, and *An*: *A. nobilis*. *Ad* 1 indicates the most common profile, observed in 211 of the 215 *A. donax* samples analyzed. Mutational and genetic differences detected between *Ad* 1 and plants with other DNA profiles are indicated in bold and starred (*). Primer sequences are listed in Table 2.

and tracing the spread of asexually reproducing invasive species, such as *A. donax*. TE-based marker systems depend on PCR to generate multilocus fingerprints that reveal polymorphisms in the insertion of members of given TE families (Baumel et al., 2002).

In this study, we designed TE-based primers for PCR amplification using published sequences (Table 2). We then used the SRAP-PCR reaction conditions and protocol described above for PCR amplification of the TE-based markers. We initially evaluated numerous primer combinations for amplification, number of bands, repeatability of results, and polymorphism using DNA from four randomly selected *A. donax* clones and the *A. formosana* and *A. nobilis* clones. From the primers tested, 14 primer sequences (Table 2) and 12 primer combinations (Table 3) generated the largest number of bands and greatest repeatability of results and polymorphism, thus were selected for DNA fingerprinting.

2.4. Size separation and scoring of amplification products

Amplicons from the SRAP and TE-based marker analyses were separated according to size on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) using a 50 cm capillary array loaded with POP7 polymer. Forward primers were labeled with the fluorescent dyes 6-FAM, HEX or NED (Applied Biosystems). PCR products with three different dyes and CST ROX 50–650 Size Standard (Bioventures, Inc., Murfreesboro, TN) were run simultaneously on the Genetic Analyzer. PCRs and marker analyses were repeated twice for each sample. Peaks (bands) were detected using GeneMapper software Version 3.7 (Applied Biosystems) and only peaks detected in both analyses were scored as present for each sample fingerprinted. Because a high frequency of *A. donax* samples exhibited a single, common multilocus DNA fingerprint, DNA was re-extracted from frozen plant samples with DNA profiles that differed from the common one. For comparison, DNA was also re-extracted from three randomly selected samples exhibiting the common profile. PCR and SRAP and TE-based marker analyses were repeated on these samples for a third time to confirm the observed genetic variation.

3. Results

A total of 185 putative clones (clumps or clusters of stems) of invasive *A. donax* were sampled in 15 states across the United States (Table 1, Fig. 1) and genetically fingerprinted with the SRAP and TE-based markers. In addition, 20 invasive clones from southern France and 10 cultivated clones (7 from the United States and 3 from the United Kingdom) of *A. donax*, and 1 clone each of commercial varieties of *A. formosana* and *A. nobilis* (Table 1) were genetically fingerprinted with the

same markers. Eight invasive clones and five cultivated clones had variegated leaves (Table 1).

A total of 185 reproducible DNA fragments were amplified in *A. donax* by the 10 SRAP primer combinations (Table 3) used in this study. The number of amplification products generated per primer pair was highly variable ranging from 5 to 40. Fragment size ranged from 50 to 400 bp. All *A. donax*, including both invasive and cultivated clones, were genetically identical based on the SRAP analysis, with the exception of one clone, which exhibited a single mutation. The invasive clone (*Ad* 2) from Texas had a 2 bp deletion that was revealed by primer combination ME2 + E39 (Table 3) and observed as a 64 bp peak instead of the 66 bp peak observed in all other plants. The mutant plant was not variegated.

A total of 164 reproducible DNA fragments were amplified in *A. donax* by the 12 TE-based primer combinations (Table 3). The number of fragments produced per primer pair was highly variable ranging from 4 to 27. Fragment size ranged from 50 to 700 bp. The TE-based markers revealed a lack of genetic variation in *A. donax* similar to the SRAP markers. All invasive and cultivated *A. donax* clones analyzed were genetically identical with the exception of three from California that exhibited different fingerprints (*Ad* 3 and *Ad* 4) attributable to single mutations detected by the primer combination RMP-R + MF8081 (Table 3). The clone with the *Ad* 3 profile exhibited deletion of a 185 bp fragment that was present in all other *A. donax* samples. The two samples with the *Ad* 4 profile were missing a 189 bp fragment that was detected in all others tested. The three variable samples were obtained from invasive clones that were not variegated.

DNA fingerprints of samples of 20 randomly selected putative clones of invasive *A. donax* in 4 populations from southern France and 10 cultivated clones, including 6 from ornamental plantings and 4 samples of varieties 'Variegata' (syn. 'Versicolor', 'Picta'), 'Golden Chain', and 'Macrophylla' were identical to the *Ad* 1 profile observed (Table 3) for the majority of plants sampled in the United States. No genetic polymorphisms were revealed in cultivated *A. donax* or invasive plants from France using the 10 SRAP and 12 TE-based primer combinations. In contrast, more than 50% of the 185 SRAP markers and 164 TE markers were polymorphic when DNA fingerprints of *A. donax* were compared with those of the cultivated species *A. formosana* and *A. nobilis*. Moreover, genetic differences between the latter two species and *A. donax* were revealed by every SRAP and TE primer combination (Table 3). The lack of genetic diversity observed within *A. donax* but large genetic differences observed between *A. donax*, *A. formosana*, and *A. nobilis* are illustrated by the traces in Fig. 2, which were generated by two primer combinations, SRAP primer combination ME2 + E39 (left panel) and TE primer combination IRP-F + BARE1 (right panel).

4. Discussion

With the exception of single mutations detected in four plant samples, *A. donax* collected from California to South Carolina and Florida exhibited no molecular genetic variation despite the wide genomic coverage of the markers used in this study. The molecular data strongly point to a single genetic clone of invasive *A. donax* in the United States. The simple mutations detected in a few sampled plants may have occurred in the invaded range following introduction of the clone into North America. The lack of genetic variation observed in this study differs from an earlier study (Khudamrongsawat et al., 2004) of *A. donax*, which revealed a moderate level of genetic variation in samples collected along the Santa Ana River in southern California. The latter study used different marker systems, i.e. isozymes and RAPDs, to assess genetic diversity than those employed here, which may account for the difference. In particular, RAPD markers are sensitive to DNA quality and PCR conditions (Vos et al., 1995) and often result in inconsistent banding patterns. Our results are consistent with the low genetic variation detected in wild populations of *A. donax* in Greece, Italy and southern France by 'The European Giant Reed (*Arundo donax* L.) Network' (Lewandowski et al., 2003). Low genetic diversity in the invaded range has also been

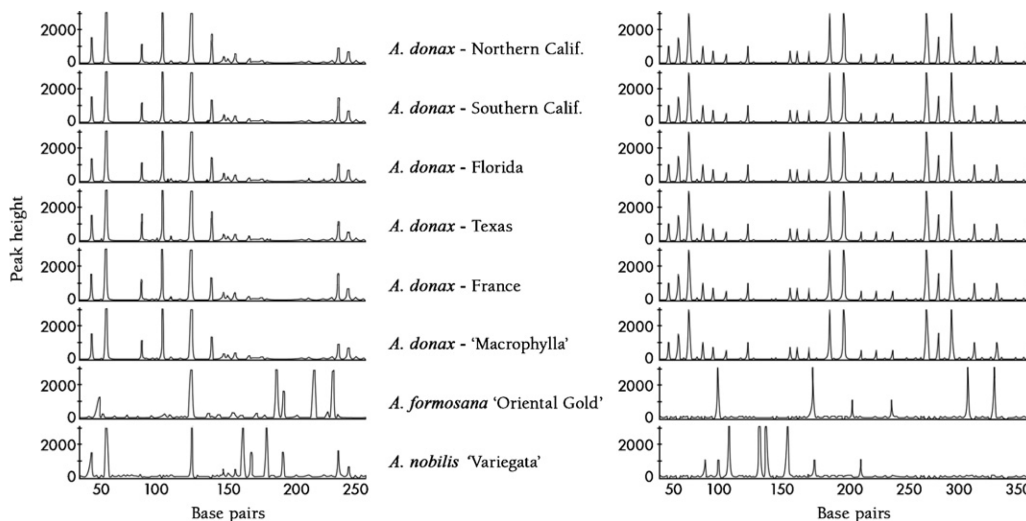

Fig. 2. DNA fingerprints generated by SRAP primer combination ME2 + E39 (left panel) and TE primer combination IRP-F + BARE1 (right panel).

observed in other exotic clonal species, including *Alternanthera philoxeroides* (Li and Ye, 2006; Wang et al., 2005), *Eichhornia crassipes* (Li et al., 2005; Ren et al., 2005) and *Fallopia japonica* (Hollingsworth and Bailey, 2000).

The genetic uniformity we detected in invasive *A. donax* within the United States may be due to genetic bottlenecks that occurred during the species' spread and cultivation around the world, and with its obligate asexual reproduction in introduced ranges. *A. donax* is considered to be native to eastern Asia (Polunin and Huxley, 1987). However, it was introduced and cultivated for thousands of years throughout Asia, southern Europe, north Africa, and the Middle East (Perdue, 1958; Zohary, 1962), and naturalized in the countries surrounding the Mediterranean Sea (Lewandowski et al., 2003). From the Mediterranean region, it is believed to have been dispersed worldwide by humans for multiple uses (Perdue, 1958). Such nested founder events, combined with the selection and clonal propagation of specific variants with desirable traits for cultivation, are likely to have resulted in substantial reductions of genetic diversity in *A. donax* during its spread. Obligate asexual reproduction in the introduced ranges of Europe and North America (DiTomaso and Healey, 2003; Dudley, 2000; Lewandowski et al., 2003) would also keep genetic diversity low.

Multiple deliberate introductions of *A. donax* into the United States have been documented. In addition to the initial introduction from the Mediterranean region into southern California in the early 1800s (Bell, 1997), rhizomes from cane plantations of southern France and others of unknown origin were also brought into California, Texas, Georgia, and Alabama for reeds for musical instruments following World War I (Perdue, 1958). Further, cultivated strains, including plant material from Iran and Afghanistan, were reported to have been introduced into Texas in the early 1940s. Following World War II, *A. donax* from France was again imported into California, and plant material from Mexico introduced into Arizona and New York (Perdue, 1958). Despite the multiple introductions of *A. donax* into the United States, the single genetic clone detected in this study suggests that the same asexual clone may have been cultivated in multiple regions of the world. Our result that samples of *A. donax* from four populations in southern France have the same DNA profile as 211 of 215 clones sampled across the United States concurs with this hypothesis.

Despite the invasiveness of *A. donax*, the species is propagated and sold horticulturally throughout the United States. A few varieties are commercially available (e.g. Loewer, 1995; Sunset, 1967). The most common is 'Variegata' (syn. 'Versicolor', 'Picta'), which has variegated leaves with white stripes and is a diminutive form of the common *A. donax* (Lewandowski et al., 2003; Perdue, 1958). 'Variegata' has been widely planted as an ornamental throughout warmer regions of the United States, and was especially popular in the Southwest where it was grown along ditches for erosion control. According to Perdue (1958), a few other varieties with variegated leaves have also been selected by horticulturalists. In this study, we sampled eight putative clones of invasive *A. donax* from California, Georgia, Louisiana, Oklahoma, and Texas that had variegated leaves (Table 1). The presence of plants in the wild with leaf variegation suggests a high rate of chloroplast DNA mutation (Tilney-Bassett, 1978) or, more likely, the presence of escapes from horticultural plantings of variegated varieties.

Arundo donax has been proposed as a candidate for biological control in the United States (Tracy and DeLoach, 1999). Our results indicating that invasive plants sampled from California to South Carolina and Florida consist of a single genetic clone suggest that *A. donax* may be an appropriate species for biological control. A major criterion used to assess the suitability of an invasive weed for classical biological control is the potential to find natural enemies that are highly host specific and damaging to the species under consideration (Goolsby et al., 2006; Tracy and DeLoach, 1999). The strategy for finding such natural enemies normally involves searching regions within the native range of the invasive weed because it is generally believed that the most specific and effective biological control agents are likely to be those that share an evolutionary history with their hosts (Williams et al., 2005). The occurrence of a single genetic clone, as detected for *A. donax* in this study, greatly simplifies assignment of invasive plants to specific source populations in the native range (*sensu* Roderick and Navajas, 2003) and thus substantially narrows the regions to be searched for natural enemies.

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