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The expansion of sterile *Arundo donax* (Poaceae) in southeastern Australia is accompanied by genotypic variation

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ABSTRACT

Reproductive modes and dispersal mechanisms shape the genotypic structure of populations which in turn influences a species' capacity for successful invasion. Understanding the limitations in a species' reproductive capacity may assist with finding the best approach to control the expansion of an invasive species. To understand the founder history against the spread of *Arundo donax*, we examined genotypic variation, using Inter Simple Sequence Repeats (ISSR) markers, and we determined ploidy and fertility in 10 single (one patch) and seven multiple (spatially distinct patches) stands scattered along three river systems in southeastern Australia. No seed was detected in any plant and shriveled anthers did not produce pollen. Somatic chromosome counts identified uneven ploidy levels ($2n = 7x = 84$), which may be the cause of sterility in *A. donax*. Of the 58 plants sampled we detected 38 genotypes, and genotypic variation was moderate to high within each river system ($G/N = 0.485–1.000$ and Simpson's $D = 0.881–1.000$). Three genotypes were found in more than one stand, suggesting that the invasive spread of *A. donax* by vegetative propagules is not a rare event. Genetically distinct genotypes were detected in all single stands. Sections of the river systems that contained multiple stands of *A. donax* revealed that each stand was a unique genotype; this may be attributable to multiple founders, somatic mutations, and/or polyploidy. Our results indicate that sterility has had little effect on retarding the dispersal of heptaploid *A. donax* in southeastern Australia, and reproduction through vegetative fragments is an effective dispersal mechanism.

1. Introduction

In plant species where reproduction is assured through vegetative propagation, a single propagule (e.g., a ramet) can initiate a new population. Many aquatic invaders use this strategy to rapidly establish populations in new habitats (e.g., *Fallopia japonica*, Hollingsworth and Bailey, 2000; *Butomus umbellatus*, Lui et al., 2005; *Alternanthera philoxeroides*, Wang et al., 2005; *Arundo donax*, Ahmad et al., 2008), albeit genetically uniform populations at first (Hollingsworth and Bailey, 2000; Ahmad et al., 2008). Range expansion and naturalization often follow as a consequence of further dispersal events (Ashton and Mitchell, 1989; Sakai et al., 2001; Kowarik and Säumel, 2008). Expansion is facilitated by a dispersal vector and may involve several different mechanisms over time (e.g., vertebrates and water in *Schinus terebinthifolius*, Donnelly and Walters, 2008). Rivers may serve as very effective dispersal corridors for species as they facilitate considerable range expansions. The range of *Tulipa sylvestris*, for example, was increased by 54 km along the Aller River in Germany by a flood event, providing an excellent example of this phenomenon (Kowarik and Wohlgemuth, 2006). Understanding the patterns of propagule movement in river systems may be crucial in stemming the colonization of new populations of invasive riparian species (Donnelly and Walters, 2008).

Characterizing the number and type of genotypes for invasive riparian species could assist with detecting mechanisms of dispersal and enable predictions of invasiveness. For example, estimates of genotypic richness (number of unique genotypes) among *Phragmites australis* subsp. *australis* stands in northeastern region of North America, provided information on the possible mechanism by which the invader dispersed over a short distance from coastline and roadways or over a long distance via a large commercially important water way, the St. Lawrence River/Great Lakes (Kirk et al., 2011). In addition, valuable information about the invasion history in a population could be evaluated by identifying unique genotypes. A complex of genotypes with high diversity implies that multiple introductions have occurred from different source regions (Novak and Welfley, 1997). Occasional hybridization and seed production (Khudamrongsawat et al., 2004) and accumulation of mutations (Gross et al., 2012) have also been suggested to contribute to high levels of variations among genotypes. In contrast, in the absence of somatic mutations, low or no genotypic variation is likely to be represented by asexuality and few founder effects (Poulin et al., 2005).

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Molecular markers are an effective and important tool to provide informative data on the levels of genotypic variation and patterns of propagule movement and invasion.

Arundo donax (giant reed, Poaceae) is a rhizomatous perennial native to Asia (Mariani et al., 2010) and it has been planted in the temperate and subtropical regions of both hemispheres (Herrera and Dudley, 2003) as an ornamental plant. This species was first brought to Australia over 150 years ago and is now naturalized and found extensively across the continent (Williams et al., 2008). Vegetative propagation through stem layering and rhizome proliferation (Boland, 2006) is believed to be the primary mode of reproduction in *A. donax* in North America (Dudley, 2000; Johnson et al., 2006) and South Australia (Williams et al., 2008). New culms sprout to form dense stands once the fragments embed into moist soil (Herrera and Dudley, 2003; Quinn and Holt, 2008) which occurs via the carriage of vegetative fragments with flood waters (Boland, 2006).

We studied fecundity, ploidy and the level of Inter Simple Sequence Repeats (ISSR) variations in *A. donax* to understand the founder history along river catchments in southeastern Australia (NSW; Hunter-Central Rivers and Namoi catchments). Since *A. donax* is a wide spread species with the potential to grow in a range of habitats, our first aim was to determine if the species is reproducing via vegetative fragments and/or seeds. We then studied the level of ploidy to evaluate the underlying factors that could disrupt seed production in southeastern Australia. Elsewhere, meiotic (Bhanwra et al., 1982; Connor and Dawson, 1993; Adams et al., 2004) or gametophytic irregularities (Mariani et al., 2010) are reported as the cause of sterility in *A. donax*. Complete or near complete asexual reproduction has been shown to have various effects on the genetic structure of *A. donax* stands within the United States (Khudamrongsawat et al., 2004; Ahmad et al., 2008) and in Europe (Lewandowski et al., 2003). Therefore, a genetic study using ISSRs was undertaken to measure the magnitude of genotypic variation in different catchment areas and to provide information on the history of dispersal. This study is the first to report on the invasion biology and genetics of *A. donax* in Australia, and our aim is to contribute to knowledge on the founder history of invasive species as well as life-history traits that can influence successful invasion, reproduction, and dispersal.

2. Materials and methods

2.1. Study species and stands

Arundo donax (Poaceae, subfamily Arundinoideae, tribe Arundineae) is a perennial grass, and mature stands are composed of culms, up to 8 m tall (Bell, 1997). Inflorescences are dense panicles with both buds and spent flowers co-occurring in many inflorescences (Jacobs and Hastings, 1993). Plants reproduce vegetatively by broken rhizomes and stems (Boland, 2006) that are dispersed by water (Herrera and Dudley, 2003; Quinn and Holt, 2008). Seed production occurs in Iran and Afghanistan (Perdue, 1958), but there is no evidence of seed set in regions where it has been introduced (Dudley, 2000; Johnson et al., 2006; Mariani et al., 2010), including South Australia (Williams et al., 2008).

In southeastern Australia, 15 stands of *A. donax* were sampled across 37,000 km^2 coastal Hunter-Central Rivers catchment. This incorporated three river systems: Upper-Hunter Valley (UPH), Lower-Hunter Valley (LOH) and Paterson/Allyn/Williams Rivers (PAW). In this region, *A. donax* is listed under the 'priority widespread weeds impacting on biodiversity' that threatens lowland red gum forests (e.g., native *Eucalyptus camaldulensis*) and lowland rainforests on floodplain (Whiffen et al., 2011). Vegetative dispersal of *A. donax* by flooding is reported in the Hunter catchment (Schneider, 2007). We also sampled two more stands at the border of Hunter and the neighboring inland Namoi catchment, which has no water connectivity with the Hunter catchment, to assess inter-catchment dispersal.

In our study, a stand was denoted as a single patch or several spatially distinct (or multiple) patches of *A. donax* in a 2 km stretch of river or habitat. Stands were separated by at least 10 km and were chosen to include a range of elevations from the top of the catchments (472 m above sea level) down to the lower end of the riparian system (Table 1; 4 m above sea level). Most stands were concentrated along creeks (Fig. 1). Some stands, such as Charlestown and Violet town in Hunter catchment, and Duri in Namoi catchment were restricted to road-side ditches that are seasonally inundated. The stands varied in size and most were small, comprising a single patch only (Table 1). The largest stands were Aberdeen in Hunter catchment, and Quirindi in Namoi catchment where *A. donax* formed multiple stands for several hundred meters along the waterways.

2.2. Fecundity

We assessed fruit production using field observations and herbarium specimens. In March 2008 and in February 2012, mature inflorescences were collected from 11 *A. donax* stands (Namoi catchment: DUR and QUI stands; Hunter catchment – UPH river system: MUR, BRC, ABR, MUS and KAY stands; – LOH river system: RAV, CFB and VIO stands; – PAW river system: PHO stand) where one inflorescence was collected per stand (n = 1–5 patches per stand). Using 20 randomly selected branches along the main rachis on each inflorescence, approximately 300 achenes (10–15 achenes per branch) were excised and viewed under a dissecting microscope to establish any evidence of developing seeds. Swollen carpels or hardened seed coats were searched for as evidence of developing seeds, and shriveled or flattened carpels were used as evidence of aborted seeds. While surveying seed production, we also dissected mature anthers to determine if they contained pollen.

2.3. Chromosome counts

Ploidy level was assessed in March 2008 and in February 2012 by obtaining root-tip meristems from fresh rhizomes of two plants from each of the stands used to determine fecundity (see Section 2.2 for stands information). The rhizomes were planted in 50-cm pots with sandy soil to encourage further rooting. A standard Feulgen staining protocol was applied to chromosome preparation of root-tip meristems following Sharma and Gill (1984). The root tips were placed in 2 mM 8-hydroxyquinoline for 3–5 h, fixed in absolute ethanol and glacial acetic acid (3:1) for at least 24 h at 4 °C, macerated in 1 M hydrochloric acid at 60 °C for 15–20 min, washed and then suspended in 45% acetic acid for 10 min. After suspension, the root tip meristems were transferred to a drop of 0.5% carbon-fuchsin solution on a microscope slide and squashed under a coverslip. Chromosome counts were made on a total of 200 cells (10 cells/10 root tips/2 plants) from each stand. An exact chromosome number could not be determined due to the small size and high number of chromosomes and, therefore an average chromosome count (±SD) per stand was calculated.

2.4. ISSR and genotypic diversity

2.4.1. DNA isolation and polymerase chain reaction (PCR) amplification

Inter-Simple Sequence Repeats (ISSRs), DNA-based molecular markers, were used to determine the levels of genotypic diversity in the three river systems (UPH, LOH and PAW). In all but one