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BIODIVERSITY RESEARCH

Genetic diversity does not affect the invasiveness of fountain grass (*Pennisetum setaceum*) in Arizona, California and Hawaii

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ABSTRACT

Pennisetum setaceum (Poaceae) is a perennial bunch grass that invaded the United States during the 20th century and is highly invasive in Hawaii, moderately invasive in Arizona, and not yet invasive in southern California. *Pennisetum setaceum* is apomictic, a condition that is normally associated with low genetic variation within populations, but even moderate levels of genetic variation among populations could account for differences in invasiveness. To determine whether genetic factors are causing the variable invasion success, we used Inter-Simple Sequence Repeat markers (ISSRs) to examine genetic variation in populations from the three areas. Screening of 16 primers revealed no genetic variation within any population or between any geographical areas, a pattern consistent with complete apomixis. Variation in invasion success appears unrelated to genetic differences among populations. Differences in the seasonal timing of rainfall among the regions may be the cause of variable invasiveness of fountain grass. Alternatively, differences in timing of introduction or duration of lag phase may have limited invasiveness in Arizona and southern California.

Keywords

Apomixis, biological invasions, genetic diversity, invasiveness, invasive species, ISSR.

INTRODUCTION

As humans move throughout the world, they purposefully or unwittingly bring many species along with them. Although many of these species may be beneficial or harmless to the new environment, small proportions of these new species become harmful pests that are economically and ecologically disastrous. As invasive species spread, they displace or destroy native species and may cause radical, and possibly permanent, changes in native ecosystems (D'Antonio & Vitousek, 1992).

Many authors have tried to pinpoint suites of traits or factors common to invasive species (Baker, 1974; Lawton & Brown, 1986; Ehrlich, 1989; Rejmánek, 1995; Daehler, 1998; Goodwin *et al.*, 1999). A growing body of work is focused on genetic traits that affect invasiveness. Some species may have 'invasive potential', and arrive with life history traits that allow them to invade an environment (Crawley, 1986; Holway, 1999; Callaway & Aschehoug, 2000; Levine, 2000; Kolar & Lodge, 2001). Such traits are present before any colonization, and could include characteristics such as a high number of offspring, enhanced competitive ability, or particular breeding systems. Successful invasions could also occur because founder populations are genetically diverse enough to undergo rapid evolution within a novel selection regime that leads to invasiveness in the new habitat (Abbott, 1992; Allard *et al.*, 1993; Thompson, 1998, 1999; Lee, 2002).

Fountain grass (*Pennisetum setaceum* (Forsk.) Chiov., Poaceae) presents an ideal study system for examining the causes of invasion success, because invasion success is variable throughout its range. *Pennisetum setaceum* is a wind-dispersed, perennial, C_4, bunch grass native to arid regions in the Middle East (Williams *et al.*, 1995; Lovich, 2000). In the Hawaiian Islands, where it was introduced as an ornamental in approximately 1917, fountain grass has rapidly established monoculture stands across lava flows on the island of Hawaii and destroyed dry forests through increasing fire frequency (D'Antonio & Vitousek, 1992; Williams *et al.*, 1995; Daehler & Carino, 1998; Cabin *et al.*, 2000). Fountain grass has also been introduced to Oahu and Kauai. Fountain grass is considered a noxious weed by the Hawaii Department of Agriculture and is one of Hawaii's most invasive horticultural plants. Fountain grass has invaded other areas with variable success, including California and Arizona (Williams *et al.*, 1995). Though the exact date of introduction(s) is unknown, fountain

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grass was probably introduced to both states during the 20th century. It is listed as one of the top eight invaders in California by the California Exotic Pest Plant Council (CalEPPC) and is considered a noxious weed in Arizona. Despite CalEPPC's listing, fountain grass in southern California is confined to roadsides and ruderal areas and has not aggressively invaded natural habitats (J. Poulin, unpublished data). In Arizona it is much more prevalent and spreading quickly (D. Foster (Saguaro National Park), pers. comm.). Eradication efforts are underway at Saguaro National Park in Tucson, AZ, but even with these efforts, populations within and surrounding the park are spreading and constantly encroaching on the removal area. Thus, fountain grass shows a gradient in invasiveness: it is a widespread, noxious weed in Hawaii; rapidly spreading in Arizona; and limited in distribution in southern California.

Many *Pennisetum* species are known to be apomictic (asexual production of seeds from mitotically derived eggs), a breeding system that results in progeny that are genetically identical to the maternal plant. Several studies indicate that *P. setaceum* is also apomictic (Narayan, 1962; Taliaferro & Bashaw, 1966; Simpson & Bashaw, 1969; Rangasamy, 1972; Lubbers *et al.*, 1994; Ozias-Akins *et al.*, 2003), but there has been little molecular work testing the level of genetic variation across large spatial scales. Several authors have cited asexuality or clonality to be a common trait of invasive species (Gray, 1986; Wilen *et al.*, 1995; Amsellem *et al.*, 2000; Hollingsworth & Bailey, 2000), yet low variability within populations may decrease fountain grass's ability to rapidly adapt to new environments, indicating that fountain grass may only be able to become invasive in habitats where it is preadapted to thrive. Few apomictic species are completely asexual (Richards, 1997), however, and multiple clones usually exist across sufficiently large geographical ranges. Because many apomicts are known to be genetically variable and widespread monoclonal species are rare (Ellstrand & Roose, 1987), it is likely that fountain grass populations in the three states have different genotypes. Populations with more invasive genotypes may account for the greater invasion success in Hawaii and Arizona. Variable invasiveness could also result from nongenetic factors such as phenotypically plastic responses to both the biotic and abiotic environment (Rice & Mack, 1991; D'Antonio, 1993; Mack & D'Antonio, 1998; Barrett, 2000; Barrilleaux & Grace, 2000; Davis *et al.*, 2000), or the presence of lag periods, in which invaders are present but not spreading (Sakai *et al.*, 2001; Mack *et al.*, 2000).

Fountain grass has been analysed for genetic variation only on the island of Hawaii, where a survey of 20 allozymes in four populations of fountain grass, including the purported population of the initial introduction, yielded no variation (D. Williams, pers. comm.). Our goal was to use hypervariable molecular markers to determine the extent of genetic variation in other areas where fountain grass has been introduced, and compare genetic variability across populations occurring in Arizona, California, and Hawaii. In view of the apomictic breeding system and the results of William's allozyme study, we expected that most variation would be detected only at the broadest geographical scale. We therefore only expect to find variation among states, rather than within states or populations.

MATERIALS AND METHODS

Methodological rationale

As *Pennisetum setaceum* may have relatively low genetic diversity, even at broad geographical scales, testing for genetic diversity will require markers that are typically highly variable. Amplified Fragment Length Polymorphisms (AFLP), Restriction Fragment Length Polymorphisms (RFLP), and Random Amplified Polymorphic DNA (RAPD) have all been successfully used to screen populations for diversity, but a more recently introduced method, Inter-Simple Sequence Repeats (ISSR), shows even higher levels of diversity than these other methods (Blair *et al.*, 1999; Monte-Corvo *et al.*, 2001; Devarumath *et al.*, 2002). While dominant markers, like ISSRs, are not suitable for genetic analyses requiring information on allele frequencies, they are appropriate in this case because we are not concerned with allele frequencies, but instead with the number of bands shared among populations and across regions. ISSRs are similar to RAPDs, but ISSR primers are longer with simple sequence repeats (SSRs), and possess a 5′ or 3′ 1–3 nucleotide anchoring sequence. ISSR primers allow for higher annealing temperatures than are common to RAPDs, and, thus, more specific binding and greater band reproducibility (Culley & Wolfe, 2001). ISSRs should be ideally suited for detecting the overall levels of variation among populations in different areas that might be associated with different levels of invasiveness.

Samples

Fountain grass spikelets were collected from 20 wild populations, located in Hawaii (3 populations), Arizona (7 populations), and California (10 populations) (Table 1). In all but population HI-1 (where seeds were mass collected without regard for maternal plant), spikelets (containing no more than one seed which may be viable or inviable) from 10 to 15 maternal plants were stored in paper bags for two to five months. Due to low viability from wild populations, 200–600 spikelets (by weight) from each wild maternal plant were planted (200 to a 10×10 cm pot) in the University of California, Irvine greenhouses. In population HI-1 seeds were planted in 46×46 cm flats. Seedlings from all populations were transplanted into individual 5×5 cm pots after three to four weeks. Plants were watered as needed and fertilized weekly.

DNA Extractions

DNA was extracted from 90 to 97 mg of fresh young leaf tissue of all plants, which were at least six weeks old, using Qiagen's DNeasy Plant Mini Kit. DNA was eluted in 200 μL of Buffer AE. DNA extracted in the same manner from two fountain grass plants grown from seed from the Park Seed Company (*Pennisetum setaceum*) was also included in each primer analysis, as well as samples from individual *Ipomopsis tenuituba* (Rydb.) V. Grant. (Polemoniaceae), *Ipomopsis aggregata* Pursh (Polemoniaceae), and wild *Zea mays* L. (Poaceae) plants for comparison.

Table 1 Locations, collection dates, planting schedule, and number of individuals tested with each primer series for all fountain grass populations

Population (State and ID)	Location	Collection season	Date planted	N sampled with 8 primers	N sampled with 18 primers
HI-1	Kaupulehu (Hawaii)	Fall 2001	2/15/02	20	10
HI-2	Diamond Head Crater (Oahu)	Fall 2002	1/22/03	9	2
HI-3	Saint Louis Heights (Oahu)	Fall 2002	1/22/03	6	2
AZ-1	Saguaro National Park	Fall 2002	1/22/03	10	2
AZ-2	Saguaro National Park	Fall 2002	1/22/03	10	2
AZ-3	Saguaro National Park	Fall 2002	1/22/03	10	2
AZ-4	Saguaro National Park	Fall 2002	1/22/03	10	2
AZ-5	Tucson	Fall 2002	1/22/03	10	2
AZ-6	Saguaro National Park	Fall 2002	1/22/03	10	2
AZ-7	Phoenix	Fall 2002	1/22/03	7	2
CA-1	San Juan Capistrano	Spring 2003	5/19/03	10	2
CA-2	Irvine	Spring 2003	5/19/03	10	2
CA-4	Laguna Hills	Spring 2003	5/19/03	10	2
CA-5	Aliso Viejo	Fall 2002	1/22/03	10	2
CA-6	Irvine	Spring 2003	5/19/03	10	2
CA-7	Laguna Beach	Spring 2002	3/15/02	10	2
CA-8	Los Angeles (near Getty Museum)	Spring 2003	5/19/03	10	2
CA-9	Camp Pendleton	Spring 2003	5/19/03	10	2
CA-10	Encinitas	Spring 2003	5/19/03	10	2
CA-11	Escondido	Spring 2003	5/19/03	10	2

ISSR protocol

Eighteen simple sequence repeats with two to three basepair anchoring sequences were used as primers to screen for genetic variability (Table 2). Primers were selected based on published ISSR studies of other grass species or general overviews of ISSR techniques (Blair *et al.*, 1999; Esselman *et al.*, 1999; Wolfe, 2000; Baumel *et al.*, 2001). Twenty-six primers were screened from these sources, and all primers with clear banding were used in the study.

For all populations except HI-1, analysis using all 18 primers was performed on only two individuals. Between six and 20 individuals were originally screened from each population for an initial group of eight primers, but because this large number of individuals did not reveal any genetic variation across eight primers, the sample size per population was lowered to allow for the testing of more primers. Typical analyses of population diversity are especially focused on detecting rare alleles, and therefore need large numbers of individuals. In this case, however, alleles found only rarely in a population cannot be causing the large-scale variation in invasion success. Assuming that variation is genetically based, variation across the states is the key factor that must be driving the variability in invasiveness observed because that is the scale at which variation in invasiveness is observed. Though the small number of individuals screened for this study cannot rule out possible rare alleles or somatic mutation events, it will detect more common differences among populations and regions that could be responsible for the patterns of invasion. By using a larger number of primers more of the genome is screened and there is a higher chance of observing variability that could affect invasiveness even if the variation is limited to a small region of the genome. Analysis using all 18 primers was performed on 10 individuals from population HI-1 because the seeds were not sorted by maternal plant.

Table 2 Primer sequences, annealing temperatures, and gel concentrations for all the primers used

Primer no.	Sequence	Annealing temp (°C)	Agarose percentage
1	$(AC)_7RG$	48	3
2	$(AG)_8YC$	48	2
3	$(AG)_8TG$	48	3
4	$(AG)_7RG$	48	2
5	$(CA)_6RY$	48	2
6	$(CA)_6RG$	48	3
7	$(CA)_6GA$	44	3.5
8	$(CT)_8RG$	43	2
9	$(GA)_9C$	48	3
10	$(GA)_7GA$	44	3.5
11	$(GT)_6YR$	48	2
12	$(GT)_6AY$	44	3
13	$(GT)_6RG$	44	2
14	$(GT)_7YG$	48	3
15	$(CTC)_4RC$	44	2
16	$(GAC)_4RC$	46	3.5
17	$(GTC)_4RC$	48	3
18	$(GTG)_4RC$	50	2

All polymerase chain reactions (PCR) were run as single primer (25 μL) reactions, using 17.025 μL ddH_2O, 2.5 μL 10X Taq buffer, 2.5 μL 2 mM dNTPs, 1.5 μL 50 μM $MgCl_2$, 1 μL 20 μM

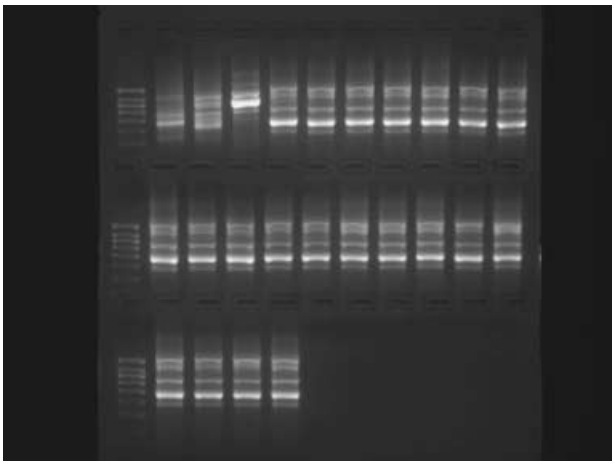

Figure 1 Gel image from primer 14. The first lane of each row is a standard 1 kb ladder. Lanes two-four in row one are the three comparison species (*Ipomopsis tenuituba* (Rydb.) V. Grant., *Ipomopsis aggregata* Pursh, and *Zea mays* L., respectively). The remaining lanes are single individuals from each of the wild collected populations (three from Hawaii, 10 from California, seven from Arizona in that order) and a single sample from a seed purchased from Park Seed Company (last lane of third row).

primer, 0.125 μL Qiagen HotStarTaq (5 U/μL) and 0.35 μL DNA per tube. Optimal annealing temperatures varied between 43 °C and 50 °C (Table 2). PCR was carried out on a Applied Biosystems GeneAmp 9700 thermocycler programmed for 15 min at 95 °C (hot start activation), followed by 35 cycles of 40 s at 94 °C, 45 s at the appropriate annealing temperature, and 1 min at 72 °C. The last cycle was 10 min at 72 °C, followed by a 4 °C soak.

Samples were run out on agarose gels of varying concentration (Table 2) in 1X TBE buffer. The gels were run at a constant voltage of 121 V for 30–75 min, depending on band separation. 1 kb ladders (GeneChoice, DNA Ladder II from PGC Scientifics Corporation) and negative controls were run on all gels. Gels were infused with ethidium bromide and visualized under UV light using an AlphaImager 3400™ (Alpha Innotech, CITY). To ensure repeatability, each primer was used in serial PCR reactions on multiple gels, and banding patterns were compared to the standard ladder to note possible banding changes. For each primer no banding changes were found between runs.

In several cases individual samples had poor PCR results for a specific primer. These samples were re-run alongside other individuals from different populations (for comparison purposes), including representatives from each of the three states.

RESULTS

In 16 of the 18 primers, banding patterns were generated from individuals with a total of 122 bands. No genetic variation was detected in any of the fountain grass samples screened (Fig. 1). The two samples grown from the Park Seed Company seeds also had banding identical to all wild collected individuals. In contrast, the samples from other species (*Ipomopsis tenuituba*, *Ipomopsis aggregata*, and *Zea mays*) all had variable banding patterns with all primers.

In the remaining two primers (no. 7 and 8), identical banding patterns, with seven and eight bands, respectively, were observed for most of the individuals. However, for primer no. 7, six individuals (13%), including plants from all three states, did not produce PCR product even after repeated reactions. Similarly, three individuals from different California populations (7%) never generated a PCR product for primer no. 8. These blank reactions could represent either genetic variability (i.e. null bands) or experimental artifacts. Given that no variability was detected with other primers and no banding was detected in these blank reactions, experimental artifact seems the most likely explanation for these results.

DISCUSSION

The molecular data from this study indicate that *Pennisetum setaceum* is generally genetically invariant in all three invaded regions studied. The lack of genetic variation is perhaps not surprising given the presumed apomictic breeding system of fountain grass. Most apomictic species, however, have some sexual capability and this breeding system is usually associated with populations of coexisting, genetically variable clones (Richards, 1997). The molecular data presented here indicate that genetic factors are unlikely to be driving the variation in invasiveness in these regions. Populations of *P. setaceum* are most likely completely apomictic and somatic mutations are probably rare, although rare sexual recombination or somatic mutation cannot be ruled out.

Some studies have called into question whether molecular diversity is a good approximation of quantitative genetic diversity (Reed & Frankham, 2001; McKay & Latta, 2002). Most of these studies have focused on allozyme analyses, which may underestimate the level of diversity, as indicated when allozyme studies and more hypervariable, DNA-based methods are compared (Esselman *et al.*, 1999). The inconsistencies between molecular and quantitative measures were diminished greatly when more variable molecular markers were employed (Merilä & Crnokrak, 2001; Reed & Frankham, 2001).

Profiles of invasive species have often focused on genetic variability as a key factor for invasion success (Abbott, 1992; Barrett, 2000; Ellstrand & Schierenbeck, 2000; Lee, 2002), but several other authors, using hypervariable molecular markers, have found successful invaders in both the plant and animal kingdoms with extremely low or no genetic variation (Williams *et al.*, 1994 — *Listronotus bonariensis* (Curculionidae); Amsellem *et al.* 2000 — *Rubus alceifolius* (Rosaceae); Hollingsworth & Bailey, 2000 — *Fallopia japonica* (Polygonaceae); Baumel *et al.* 2001 — *Spartina anglica* (Poaceae)). Invaders with lower levels of genetic variation in their introduced range than in their native range have often been observed in different types of organisms (Baker & Moeed, 1987; Novak & Mack, 1993; Neuffer & Hurka, 1999; Tsutsui *et al.*, 2000). This low level of diversity is usually attributed to founder effects and may be central to explaining the success of some invaders (Tsutsui *et al.*, 2003). Other invaders have more consistent low genetic diversity. The Argentine stem weevil shows extremely low diversity in both native and introduced ranges

(Williams *et al.*, 1994). Until it is possible to collect fountain grass seeds from its native range in the Middle East (including Libya, Jordan and Syria), it will remain unclear if low levels of genetic variation are typical of fountain grass populations worldwide or represent founder effects.

The lack of molecular variation detected using ISSR markers suggests that either abiotic or biotic environmental factors or variation in lag phases among the three regions may be influencing invasion success in fountain grass. Many authors have stressed the importance of environmental factors along with phenotypic plasticity of traits associated with invasiveness in the spread of invaders (Rice & Mack, 1991; Lodge, 1993; Schweitzer & Larson, 1999; Weber & D'Antonio, 1999; Barrilleaux & Grace, 2000), including fountain grass (Williams & Black, 1996). In many of these cases variability in field settings and a lack of information on genetic diversity makes it difficult to determine whether genetic variation among populations or environmental factors are driving the invasion. In fountain grass, where genetic variation has not been detected, environmental variation is the more likely cause of variable invasiveness. The obvious differences in seasonal rainfall and temperature extremes in Hawaii, Arizona and southern California may be largely responsible for the variable invasiveness in this species. Particularly notable are the short, single wet season in southern California, the longer wet season (with occasional rain outside of the typical season) in Hawaii, and both summer and winter rains in Arizona. Invasion in southern California may be unlikely because the rainy season is the coldest portion of the year, a period when C_4 species are least able to take advantage of the moisture. CalEPPC's other most invasive wildland pest plants are C_3, except for *Carpobrotus edulis* (ice plant), which is CAM-inducible.

High levels of phenotypic plasticity in Hawaiian populations of fountain grass have been well documented for both morphological and physiological traits (Williams & Black, 1993, 1996; Williams *et al.*, 1995; Goergen & Daehler, 2001). Such high phenotypic plasticity could account for the very invasive behaviour of Hawaiian populations. Additionally, *P. setaceum* is polyploid (typically triploid $2n = 27$, with rare occasions of hexaploid individuals $2n = 54$) (Simpson & Bashaw, 1969; Rangasamy, 1972), which may increase the plasticity of the species. Polyploidy can maintain intraindividual genetic diversity through duplicated loci, even when interindividual diversity is low or nonexistent. In William's unpublished allozyme survey fixed heterozygous patterns were common, suggesting intraindividual variation may be important in this species (D. Williams, pers. comm.). This variation may be especially important for adaptation to novel environments in an asexual species. The variation in invasiveness may be explained if environmental characteristics common to Arizona and California are outside the plastic response of this species and could be either limiting (in Arizona) or stopping (in California) spread in these areas. Alternately, differences in date of introduction or in length of lag phase could be causing the variable level of invasiveness, especially as length of lag phases can vary widely across habitats (Ellstrand & Schierenbeck, 2000).

If environmental variation is a factor in the invasion of fountain grass, then global climate change could also affect the invasion trajectory for fountain grass as it has in other species (Curtis *et al.*, 1994; McKee & Richards, 1996). Because *P. setaceum* is a C_4 species, more summer rainfall in southern California would likely have a dramatic effect on the invasion rate in that area, and allow this species to spread as rapidly in southern California as it has in Hawaii and Arizona.

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