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Genetic analysis of native and introduced populations of the aquatic weed *Sagittaria platyphylla* – Implications for biological control in Australia and South Africa

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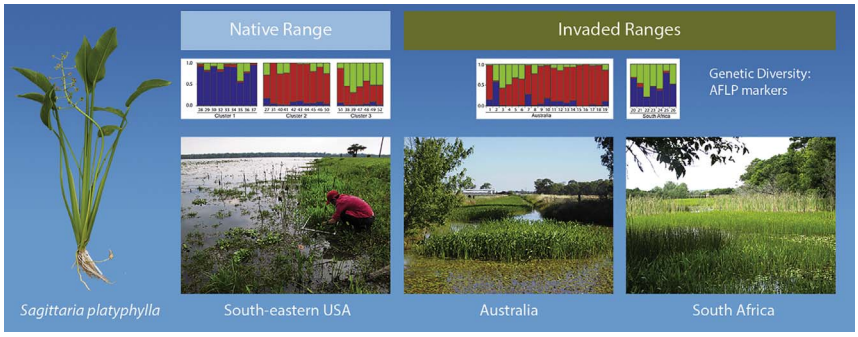
GRAPHICAL ABSTRACT



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ABSTRACT

Sagittaria platyphylla (Engelm.) J.G. Sm. (Alismataceae) is an emergent aquatic plant native to southern USA. Imported into Australia and South Africa as an ornamental and aquarium plant, the species is now a serious invader of shallow freshwater wetlands, slow-flowing rivers, irrigation channels, drains and along the margins of lakes and reservoirs. As a first step towards initiating a classical biological control program, a population genetic study was conducted to determine the prospects of finding compatible biological control agents and to refine the search for natural enemies to source populations with closest genetic match to Australian and South African genotypes. Using AFLP markers we surveyed genetic diversity and population genetic structure in 26 populations from the USA, 19 from Australia and 7 from South Africa. Interestingly, we have established that populations introduced into South Africa and to a lesser extent Australia have maintained substantial molecular genetic diversity comparable with that in the native range. Results from principal coordinates analysis, population graph theory and Bayesian-based clustering analysis all support the notion that introduced populations in Australia and South Africa were founded by multiple sources from the USA. Furthermore, the divergence of some Australian populations from the USA suggests that intraspecific hybridization between genetically distinct lineages from the

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native range may have occurred. The implications of these findings in relation to biological control are discussed.

1. Introduction

Aquatic macrophytes are recognized as causing some of the world's most intractable weed problems through their impact upon the health of animals, humans, and aquatic ecosystems, as well as the human uses of these systems (Hussner, 2012). These species are often more difficult to manage than terrestrial weeds due to the limited options available for the safe use of chemical herbicides in aquatic environments, increasing regulatory restrictions on herbicide use in and around waterways, and the need for specialized equipment to undertake mechanical and chemical control (Charudattan and Riches, 2001). Conversely, aquatic weeds are often more successfully managed using classical biological control (biocontrol) than their terrestrial counterparts (Paynter et al., 2012). Some of the most notable successes are *Alternathera philoxeroides* (Mart.) Griseb. (alligator weed), *Eichhornia crassipes* (Mart.) Solms (water hyacinth), *Lythrum salicaria* L. (purple loosestrife), *Pistia stratiotes* L. (water lettuce) and *Salvinia molesta* Mitchell (giant salvinia) (see Julien and Griffiths (1998), McFadyen (2000) and references therein).

Levels of genetic diversity in weed populations and compatibility of biocontrol agents to invasive genotypes are two factors critical to the success of biocontrol (Gaskin et al., 2011; Müller-Schärer et al., 2004; Nissen et al., 1995). Hence, molecular approaches are being increasingly utilized during the initiation phase of biocontrol research and prior to the release of agents to address key issues such as (1) accurate taxonomic identification of the target weed including the identification of novel hybrids (Hufbauer, 2004; O'Hanlon et al., 2000), (2) comparison of genetic structure within and among populations between native and invaded ranges or between different invaded habitats (Cuda et al., 2012), and (3) pinpointing the origin(s) of invasive populations where compatible natural enemies might be found (Müller-Schärer et al., 2004).

In this study, we utilized molecular approaches to compare genetic diversity and population genetic structure between native and invasive populations of the aquatic monocot *Sagittaria platyphylla* (Engelm.) J.G. Sm. (Alismataceae) (delta arrowhead) to evaluate the likelihood of successful biocontrol. This species is an emergent, herbaceous perennial endemic to southeastern and central USA and Mexico (Keener, 2005). It was deliberately introduced to Australia as an ornamental plant during the early 19th century and is now an aggressive invader of shallow waterways (AWC, 2012). It occupies a broad geographical area in Australia ranging from temperate to sub-tropical climates, invading both natural and constructed aquatic environments and is especially problematic in irrigation canals where extensive populations increase siltation, reduce water flow and cause periodic flooding (Adair et al., 2012). In South Africa, *S. platyphylla* was first recorded at the Grahamstown Botanic Gardens in 1999 (Lesley Henderson, Southern African Plant Invaders Atlas, personal communication 2012). Further plantings were recorded in the Pretoria, Pietermaritzburg and Durban Botanic Gardens in 2010 and most likely provided the source for incursions elsewhere. It is now regarded as a new and emerging weed with naturalized populations occurring in KwaZulu Natal and the Eastern and Western Capes (H. Sithole, SANBI, 2011, personal communication). Invasive populations of this weed are difficult and costly to manage and as a consequence, *S. platyphylla* is under consideration as a target for biocontrol (Sagliocco et al., 2007). As *S. platyphylla* had never been the subject of a biological control program elsewhere, little was known about the natural enemies associated with the species or of its population genetics in its native and invaded ranges. Hence, we conducted a concurrent study throughout the USA, Australia and South Africa to catalogue the flora and fauna associated with *S. platyphylla* (Kwong et al., 2014) and collect plant samples for the genetic analysis. We used Amplified Fragment Length Polymorphism markers (AFLPs) to compare relative levels of genetic diversity and assess population genetic structure in native USA and introduced Australian and South African populations to make explicit recommendations for future biocontrol research. We primarily addressed four questions: (1) Is genetic diversity in the invasive range comparable to that in the native range? This will help us assess the genetic complexity of the invaded ranges with respect to the likely success of biocontrol agents since several studies have shown that some pathogens and herbivores can be adapted to specific genotypes or populations of their host plants (e.g. Goolsby et al., 2006). Conversely, the success rate of biocontrol is generally higher for invasive weed populations with low genetic diversity as there is less risk of "resistant" weed genotypes replacing "susceptible" genotypes that have been controlled by highly-adapted biocontrol agents (e.g. Evans et al., 2005); (2) Are invasive Australian and South African populations genetically divergent from native populations? This will help us determine the likelihood of finding pre-adapted prospective biocontrol agents to attack invasive genotypes; (3) Do Australian genotypes differ from South African? This will help determine if prospective biocontrol agents can be shared between these countries; (4) Can we identify the native sources of introduced populations? This will help potentially identify locations and environments in the USA where more effective biocontrol agents for Australia and South Africa *S. platyphylla* may reside.

2. Materials and methods

2.1. Study species

Sagittaria platyphylla is a diploid (2n = 22) (Baldwin and Speese, 1955) species with monoecious racemose inflorescences bearing 3–8 whorls of unisexual flowers (Keener, 2005). The species has a complex reproductive strategy that promotes reproductive assurance including a prolonged 5–6 month flowering season, incomplete self-incompatibility and avoidance of pollen limitation through extra-gynoecial pollen tube growth (Hoebee and Edwards unpublished report). Autogamy (self-fertilization within an inflorescence) is avoided by temporal separation of male and female flowers although partial overlap of these flowering phases has been observed (Hoebee and Edwards unpublished report). Geitonogamy among clones (ramets) of the same genetic individual (genet) may occur as has been observed in the closely related *Sagittaria isoetiformis* J.G. Smith and *Sagittaria teres* S. Watson (Hoebee and Edwards unpublished report). *Sagittaria platyphylla* produces aggregate fruit of one-seeded carpels (Adair et al., 2012) with an average of 700 and 730 achenes per female flower in Australian and South African invasive plants respectively (Kwong et al., 2017). Achenes can germinate immediately or remain dormant for several years until conditions are favorable (R. Kwong unpublished data).

Vegetative reproduction occurs through the formation of ramets and tubers at the terminal end of stolons (Adair et al., 2012), and in Australia, uncontrolled vegetative growth enables populations to rapidly expand into dense stands that dominate shallow water bodies. Preliminary genetic assessments indicated that these stands are actually comprised of multiple clones approx. 10 m apart (C. Chong unpublished). Multiple genotypes can occur in infestations that have established following a water draw-down event since this provides favorable conditions for the mass germination of achenes from a genetically-diverse seedbank. Dispersal is primarily hydrochorous with achenes floating for up to seven days (R. Kwong personal observation) although ingestion of achenes and adherence to water birds may

contribute to some long distance dispersal (Adair et al., 2012).

2.2. Sampling

Sagittaria platyphylla was sampled across Australia, South Africa and the USA (Table 1, Fig. 1) from natural (rivers, creeks, swamps) and artificial (roadside drainage ditches, irrigation canals, ornamental ponds) aquatic systems. Samples were collected from sites covering the widest geographic distribution of both the native and introduced ranges to capture the broadest possible range of genetic diversity. At each location, young and disease-free leaves were collected from plants approx. 10 m apart with the number of samples taken dependent on the size of each population to avoid oversampling in smaller sites. Additional samples from northern Victoria, Australia collected by C. Chong were also included (Broadhurst and Chong, 2011). Leaves were wiped to remove moisture and extraneous material, and a 5 cm^2 section taken and individually stored in silica gel. Populations were geo-referenced and basic site description data collected. Samples collected in the native range were taxonomically verified and accessioned into the University of West Alabama Herbarium.

Table 1
Sampling locations, numbers of samples (N) and mean (standard error) estimates of within population genetic diversity in *Sagittaria platyphylla* native populations in the southern USA and introduced populations in Australia and South Africa based on a survey of Amplified Fragment Length polymorphism variation at 140 loci on 584 samples. Genetic diversity indices include the proportion of polymorphic loci at the 5% level expressed as a percentage (*PLP*) and expected heterozygosity under Hardy-Weinberg genotypic proportions (*Hj*); standard error (s.e).

Pop[A]	Site Reference	Location	State[B]	Lat, Long[C]	N	*PLP*	*Hj* (s.e.)
Australia							
1	240211-2	Albany	WA	−35.014, 117.895	13	70.0	0.27 (0.02)
2	250512	Melbourne	VIC	−37.814, 144.976	6	82.1	0.33 (0.02)
3	Chong (Site A, B, C)	Barmah Creek	VIC	−35.968, 144.939	7	76.4	0.30 (0.02)
4	Chong (Site F)	Little Budgee 1	VIC	−35.857, 145.007	7	82.1	0.29 (0.01)
5	Chong (Site G)	Little Budgee 2	VIC	−35.883, 145.003	16	80.0	0.29 (0.01)
6	Chong (Site M)	Moira Lake	VIC	−35.955, 144.951	6	74.3	0.25 (0.01)
7	150211-2	Numurkah	VIC	−36.106, 145.362	14	75.7	0.29 (0.02)
8	CAN 770514	Ovens River, Bundalong	VIC	−36.049, 146.188	7	74.3	0.25 (0.01)
9	090311-1	Deniliquin/Finley	NSW	−35.555, 145.030	12	80.7	0.26 (0.01)
10	150211-1	Shepparton	VIC	−36.355, 145.402	16	69.3	0.26 (0.02)
11	100310_3	Brisbane	QLD	−27.483, 152.903	5	75.0	0.26 (0.01)
12	JM050411	Binna Burra/Bangalow	NSW	−28.710, 153.487	16	85.0	0.29 (0.01)
13	110310_1	Eumundi/Cooroy	QLD	−26.479, 152.956	9	78.6	0.22 (0.01)
14	TI220311	Bulahdelah	NSW	−32.407, 152.200	6	78.6	0.33 (0.01)
15	290311-1	Woongarah/Wyong	NSW	−33.244, 151.490	5	68.6	0.27 (0.02)
16	300311-1	Penrith	NSW	−34.740, 150.686	5	76.4	0.28 (0.01)
17	300311-2	North Richmond	NSW	−33.585, 150.723	7	75.0	0.30 (0.02)
18	310311-1	Lane Cove National Park	NSW	−33.792, 151.151	5	71.4	0.31 (0.02)
19	RJC1-050511	Thirlmere	NSW	−34.205, 150.573	7	85.7	0.32 (0.01)
South Africa							
20	NA	Grahamstown	EC	−33.318, 26.522	15	79.3	0.28 (0.01)
21	NA	Maden	EC	−32.737, 27.298	7	80.7	0.33 (0.01)
22	NA	Farningham Ridge	KZN	−29.836, 30.871	12	86.4	0.30 (0.02)
23	NA	Wensleydale	KZN	−29.601, 30.409	10	88.6	0.34 (0.01)
24	NA	Keisie	WC	−33.685, 19.984	12	87.1	0.29 (0.01)
25	NA	Stellenbosch	WC	−33.963, 18.926	10	81.4	0.29 (0.02)
26	NA	Northern Paarl	WC	−33.700, 18.982	13	86.4	0.30 (0.01)
USA							
27	230810-2	Warsaw/Boligee	AL	32.792, −88.029	21	93.6	0.35 (0.01)
28	080912-1	Creola	AL	30.942, −88.024	6	69.3	0.28 (0.02)
29	080912-1	Guntersville Reservoir	AL	34.367, −86.225	18	77.9	0.25 (0.01)
30	030912-2	Black Warrior River, Tuscaloosa	AL	33.437, −87.380	13	85.0	0.29 (0.02)
31	120810-1	McGehee	AR	33.564, −91.384	16	61.4	0.21 (0.02)
32	130912-2	Pine Bluff	AR	34.252, −91.983	10	84.3	0.28 (0.01)
33	130912-1	Gould, AR	AR	34.049, −91.642	9	75.7	0.25 (0.02)
34	140912-1	Pinnacle Mountain State Park	AR	34.847, −92.464	4	63.6	0.28 (0.02)
35	180912-1	Caddo Lake	TX	32.720, −94.116	20	83.6	0.31 (0.02)
36	250812-1	Richmond Hill	GA	31.955, −81.320	18	80.0	0.24 (0.01)
37	120912	Yazoo NWR	MS	33.125, −91.003	14	71.4	0.24 (0.01)
38	040810-1	Tara Wildlife Reserve	MS	32.479, −91.062	19	83.6	0.28 (0.01)
39	060810-1	Winnsboro	LA	32.149, −91.705	19	82.9	0.28 (0.01)
40	060810-2	Cheniere Brake	LA	32.458, −92.203	8	87.1	0.30 (0.01)
41	060810-3	Monroe	LA	32.556, −92.075	9	87.1	0.30 (0.01)
42	060810-4	Black Bayou	LA	32.604, −92.049	8	70.7	0.23 (0.01)
43	090810-1	Senatobia	MS	34.643, −89.971	14	70.7	0.26 (0.02)
44	110810-1	Stoneville	MS	33.422, −90.905	14	64.3	0.20 (0.02)
45	180810-1	Reelfoot Lake	TN	36.468, −89.316	16	75.0	0.28 (0.01)
46	180810-1	Sunk Lake	TN	35.710, −89.738	7	69.3	0.21 (0.01)
47	190811-1	Hackberry	LA	29.994, −93.358	7	81.4	0.36 (0.01)
48	200811-1	Moss Bluff	LA	30.294, −93.234	10	92.9	0.36 (0.01)
49	220811-1	St Martinville	LA	30.222, −91.906	14	90.7	0.36 (0.01)
50	250811-1	New Orleans	LA	30.261, −89.786	17	94.3	0.32 (0.01)
51	100811-1	Lewisville	TX	33.069, −96.959	14	90.7	0.30 (0.01)
52	130811-1	San Marcos River	TX	29.876, −97.932	11	92.1	0.36 (0.01)

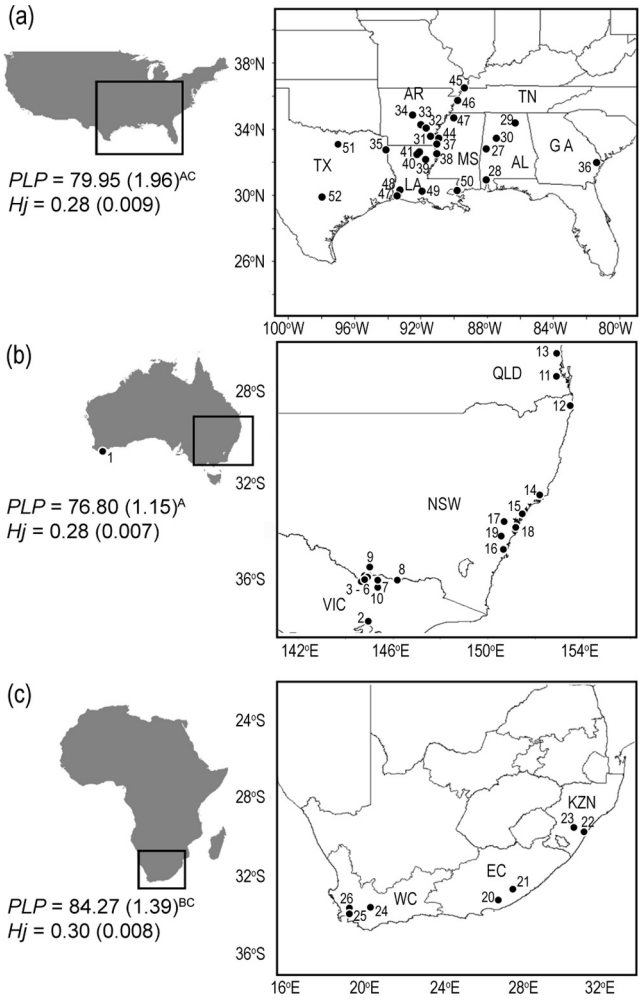

Fig. 1. Site location and genetic diversity of *Sagittaria platyphylla* populations sampled in (a) the native USA and invaded ranges in (b) Australia and (c) South Africa. Values of diversity indices* shown are mean (SE) and values marked with different letters are significantly different at $P = 0.05$ level (Kruskal-Wallis one-factorial ANOVA and to multiple Mann Whitney U-tests with Bonferroni correction for *post hoc* analyses. Refer to Table 1 for sampling information. * *PLP,* percentage of polymorphic loci; *Hj*, gene diversity.

2.3. DNA extraction and AFLP amplification

Total genomic DNA was extracted according to a modified protocol of Blundell et al. (2010) from approx. 3 mg of leaf tissue ground to a fine powder using 3 mm tungsten carbide beads in a Retsch MM300 mixer mill, and quantified using a Bio-tek Power Wave HT-1 (Millennium Science) plate reader. Only four of the 42 microsatellite markers specifically developed for *S. platyphylla* were polymorphic. Polymorphism was extremely low in these markers with only 2–4 alleles detected across a diverse sample of 48 plants from different populations, a finding that reflects limited diversity previously observed using allozymes (Hauber and Lege, 1999). Consequently, we opted to use Amplified Fragment Length Polymorphisms (AFLPs) as described in Vos et al. (1995) with the following modifications: 400–500 ng of DNA were digested with *Pst*I/*Mse*I for 2 h at 37 C, *Pst*I/*Mse*I adaptors were ligated on both ends of the DNA fragments followed by preselective amplification using *Pst*I + *A* and *Mse*I + *G* or *Mse*I + *C* primers. Six primer combinations were tested and four used due to their higher polymorphism and repeatability (*Pst*-AC/M-GAG, *Pst*-AG/*M*-GAG, *Pst*-AC/*M*-CTC, *Pst*-AG/*M*-CTC). Selective amplification was carried out with *Pst*I + 2 fluorescently labeled and *Mse*I + 3 unlabeled primers and visualized on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc.) using an ABI GS600LIZ internal ladder. To ensure reproducibility of our results a positive control was used for each plate. In addition, DNA was extracted twice for a subset of samples which were then amplified twice on different PCR machines. Fragments less than 100 base pairs were excluded, bins were manually set to a width of 1 base pair, bands with peak heights < 70 relative fluorescent units (rfu) were ignored and bins with > 10% mismatch errors were eliminated. Fragments were assessed for polymorphism with 140 markers being considered sufficiently variable for analysis. These were scored as presence/absence characters using GeneMapper® Version 4 (Applied Biosystems Inc.) and a binary matrix constructed for analyses.

2.4. Genetic diversity in the USA (home) and invaded ranges

AFLP-SURV 1.0 (Vekemans, 2002) was used to estimate allele frequencies using a Bayesian approach that assumes Hardy-Weinberg genotypic proportions with a non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999). These frequencies were then used to calculate the proportion of polymorphic loci at the 5% level expressed as a percentage (*PLP*), and gene diversity (*Hj*), which is analogous to the expected heterozygosity (H_e) under Hardy-Weinberg equilibrium, were estimated for USA, Australian and South African populations. Differences in these genetic diversity measures between invasive Australian and South African and native USA populations were tested using a Kruskal-Wallis one-factorial ANOVA (GenStat, 2013) and multiple Mann Whitney U-tests with Bonferroni correction for *post hoc* analyses.

2.5. Population structure in the USA

Given the complex temporal and spatial nature of hydrologically-dispersed organisms and our limited understanding of invasion history we used several approaches to assess population genetic structure. Bayesian assignment using STRUCTURE 2.3.2 (Pritchard et al., 2000) was used to infer the number of genetic clusters (K) present in the USA samples without prior knowledge of population affinities. Five runs with a 50,000 burn-in followed by 500,000 MCMC for $K = 1$–10 using both Admixture and No Admixture models, a uniform prior for α, an initial α of 1 and allele frequencies correlated among populations were generated. The optimal number of *K*-clusters was determined with the *ad hoc* statistic Δ*K* (Evanno et al., 2005) using Structure Harvester version 0.6.93 (Earl and vonHoldt, 2012). STRUCTURE outputs for each *K* in each analysis were summarized in CLUMPP version 1.1.2 (Jakobsson and Rosenberg, 2007) using the Greedy algorithm with random input and 1000 permutations and plotted.

Pairwise relatedness between individuals (r) was estimated using the Taylor expansion (Lynch and Milligan, 1994) in AFLP-SURV. The relatedness matrix generated was used to extract and plot the first two principal coordinates in GenStat 18th edition (GenStat, 2013).

The PopGraph package in GeneticStudio version 1.3.1 (Dyer and Nason, 2004) was used to assess connectedness among populations and distribution of genetic variation among USA populations and among USA, Australian and South African populations.

TESS version 2.3.1 (Chen et al., 2007), which unlike STRUCTURE incorporates spatial information, was run with a 25,000 burn-in followed by 250,000 sweeps for each *K* from 2 to 26 using the CAR admixture algorithm based on the Delaunay tessellation and an interaction parameter of 0.6. The deviance information criterion (DIC) was calculated for each of the 50 replicates for each *K*. The top 10% of these runs (i.e. those with the lowest DIC) were retained and the average of these plotted to identify the best *K* for this data (Chen et al., 2007). Runs were aligned in CLUMPP version 1.1.2 (Jakobsson and Rosenberg, 2007) using the Greedy algorithm with random input and 1000 permutations.

Using AFLPOP version 1.1 (Duchesne and Bernatchez, 2002) we assessed the probability of incorrectly assigning US samples to source populations by simulating 1000 genotypes over 10 iterations followed

by the re-allocation procedure to assign US samples to their most likely source population. For these analyses we replaced alleles frequencies of 0 with 1/(sample size + 1) and used a minimum maximum-likelihood difference (MLD) of 0 (individuals are assigned with the highest probability) or 1 (individuals were assigned if their probability of belonging to a population was at least 10X higher than the probability of their belonging to another population). We then separately assigned Australian and South African samples to the US source populations and to the $K = 3$ determined by STRUCTURE (see below) using the same parameters.

2.6. Genetic comparison of home and invaded ranges and origins of invasion

A hierarchically-nested AMOVA model calculated from the pairwise squared Euclidean distances between individuals was used to examine how molecular variation was partitioned among provenances (continents) relative to the total populations (ϕ_{RT}), among populations within continents (ϕ_{PR}) and within populations (ϕ_{PT}) using GenAlEx 6.501 (Excoffier et al., 1992; Peakall and Smouse, 2012) with tests of significance based on 999 random permutations. AMOVA for each continent was also conducted separately to characterize genetic structure among and within populations (ϕ_{PT}). The USA samples were coded according to their most likely cluster of origin previously identified by STRUCTURE (i.e. $K = 3$, see below) and used as 'learning samples' against which the ancestry of Australian and South African samples were inferred with the USEPOPINFO model within STRUCTURE. Finally, associations among the USA, Australian and South African samples and populations were assessed using the PCoA and graph-theory approaches described above.

3. Results and discussion

3.1. Genetic diversity in the USA (home) and invaded ranges

A total of 584 individuals from Australia (19 populations), South Africa (7 populations) and the USA (26 populations) were genotyped with genetic diversity measures varying within and among populations and continents (Table 2, Fig. 1). For example, in Australia the proportion of polymorphic loci (*PLP*) ranged from 69–86% compared with 79–89% in South Africa and 61–94% in the USA. Australian populations displayed significantly lower *PLP* than South Africa, although no differences were detected between Australia and the USA and the USA and South Africa (Kruskal-Wallis $\chi^2_{(2)} = 6.2$, $P = 0.045$; Supplementary Material Table S1). Gene diversity (*Hj*) did not differ significantly among the three continents (*Hj*; $\chi^2_{(2)} = 2.42$, $P = 0.3$). The first three PCoA axes for all native and invasive plants accounted for 8.4%, 6.5% and 4.1% of the total variation respectively (Fig. 2). USA and Australian individuals mostly separated into two clusters along PCoA2 with the South African plants being distributed among the USA samples. Hierarchical AMOVA of the native and invaded ranges reflected significant genetic structuring at all levels of this analysis (Table 2) with 9% of the variation ($P = 0.001$) distributed among the three continents and 23% of the variation ($P = 0.001$) among populations within continents. When continents were examined separately, among population differentiation was greatest in the USA (30%), and lowest in South Africa (16%). In all three continents, the majority of genetic variation was retained within populations, namely 70% in the USA, 82% in Australia and 84% in South Africa.

These results show that genetic diversity in *S. platyphylla* in introduced ranges is comparable with that in the native range, suggesting that founding populations were diverse and/or that multiple introductions from different native sources have generated genetically mixed populations within the invaded range. The introduction history of *S. platyphylla* to Australia is poorly known but the species was a popular aquarium and ornamental pond plant as early as 1933 (NLA, 2014). Consequently, it is difficult to determine whether invasive populations were originally comprised of diverse lineages or whether the diversity detected here has been generated since introduction.

3.2. Population structure in the USA

Based on ln(P)*K*, STRUCTURE determined $K = 2$ as the most probable number of clusters for native USA populations whereas for ΔK it was 3 (Fig. 3a and Supplementary material Fig. S1a) for both the Admixture and No Admixture models (data not shown for latter model). Assignment of $K = 2$ (Supplementary material Fig. S2) was poorly aligned with any geographic structure whereas $K = 3$ was more closely associated with geographic groupings based on expectations of a hydrologically-dispersed organism such as *S. platyphylla* (Level II watershed regions, Fig. 4). Many $K = 3$ populations were strongly assigned by STRUCTURE to Clusters 1 (blue) and 2 (red) based on > 70% identity (range 70–96%) whereas Cluster 3 (green) populations were a mix of Clusters 2 and 3. Although regional distribution of $K = 3$ found representatives of each Cluster in each watershed (Fig. 4), within Clusters linear distributions associated with expected downstream directional flow were evident (e.g. the Arkansas-White-Red Region Pops 34, 32 and 33, Cluster 1, blue; Lower Mississippi Region Pops 45, 46 and 43, Cluster 2, red). The majority of populations that occurred downstream from here were assigned to the 'mixed' Cluster 3 (green; Pops 38, 39, 47–49). Assessments of $K = 4$–5 failed to better describe the data (Supplementary material Fig. S2).

The first two principal coordinates (PCoA) axes explained 17.3% of the genetic variation present and showed similar differentiation among individuals to that observed among populations in STRUCTURE (Fig. 3b). Cluster 1 (blue) individuals were primarily separated in negative PCoA2 space while there was also some separation between

Table 2
Hierarchical nested analysis of molecular variance (AMOVA; Excoffier et al., 1992) conducted on 140 Amplified Fragment Length Polymorphism (AFLP) loci from 52 *Sagittaria platyphylla* population from native (USA) and introduced (Australia and South Africa) ranges. The degrees of freedom (df), sum of squares (SS), mean sum of squares (MS), variance,% variance and ϕ statistics are shown. Tests of significance were based on 999 permutations. $^{*}P < 0.001$.

Source of variation	df	SS	MS	Variance	% Var	Statistic
Native + introduced ranges						
Among continents	2	859.69	429.85	2.12	9	$\phi_{RT} = 0.091$*
Among populations within continents	49	3676.78	75.04	5.31	23	$\phi_{PR} = 0.253$*
Within populations	532	8367.93	15.73	15.73	68	$\phi_{PT} = 0.321$*
Total	583	12904.40		23.16	100	
Native (USA) range						
Among populations	25	2589.25	103.57	6.82	30	
Within populations	310	4912.86	15.85	15.85	70	
Total	335	7502.11		22.67	100	$\phi_{PT} = 0.301$*
Invasive (Australia) range						
Among populations	18	772.72	42.93	3.18	18	
Within populations	150	2242.31	14.95	14.95	82	
Total	168	3015.03		18.13	100	$\phi_{PT} = 0.175$*
Invasive (South African) range						
Among populations	6	314.81	52.47	3.18	16	
Within populations	72	1212.76	16.84	16.84	84	
Total	78	1527.57		20.02	100	$\phi_{PT} = 0.159$*

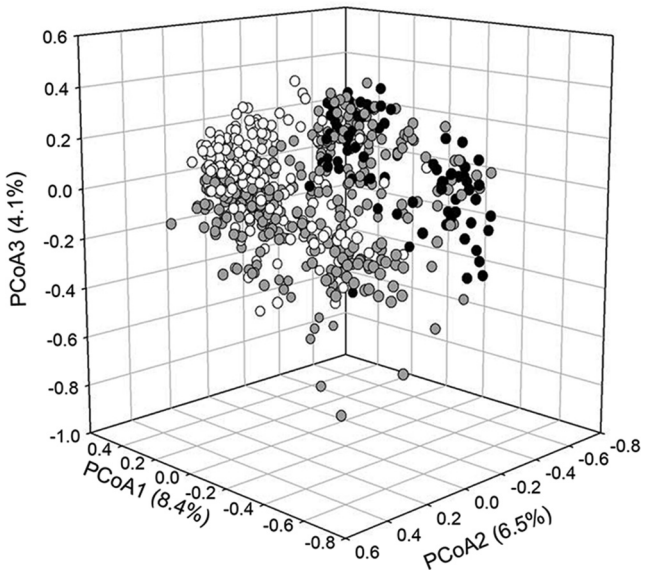

Fig. 2. Principal coordinate analysis based on Lynch and Milligan's (1994) pairwise comparisons of relatedness (r) among USA (grey circles), Australian (open circles) and South African (black circles) individuals.

samples from Clusters 2 (red) and 3 (green) along the PCoA1 axis. The Genetic Studio PopGraph package produced a complex closed and highly connected typology linking many populations in the clusters identified by STRUCTURE such as Cluster 2 Pops 31 and 44–46 and Cluster 3 Pops 47–49 (Fig. 3c). Some unexpected geographic linkages such as Cluster 1 Pop 35 in eastern Texas being linked to Alabama Pops 28 and 29, Mississippi Pop 37 and Georgia Pop 36 suggest some long distance dispersal across catchments. The mean DIC for the top 10% of TESS runs for each K decreased sharply but only reached a plateau at $K = 11$ (Supplementary material Fig. S1b). There was no clear support for $K = 3$ although the slope from $K = 2$ to $K = 3$ was slightly steeper than for other values of K. It is unclear whether this result reflects a genuine lack of structure or that the assumptions underlying TESS are inappropriate for this species (Guillot, 2009).

AFLPOP simulations indicated that the majority of samples could be reliably assigned to their source populations (> 95%) and reallocation of samples found that the majority (83.3%) were correctly assigned with a further 8.3% assigned to a different population and 8.3% not allocated to a population (Fig. 5).

3.3. Sources of introduction into Australia and South Africa

Genetic studies can be used to prioritize areas for collecting biocontrol agents by pinpointing possible geographic and/or genetic sources of origin of invasive populations (Estoup and Guillemaud,

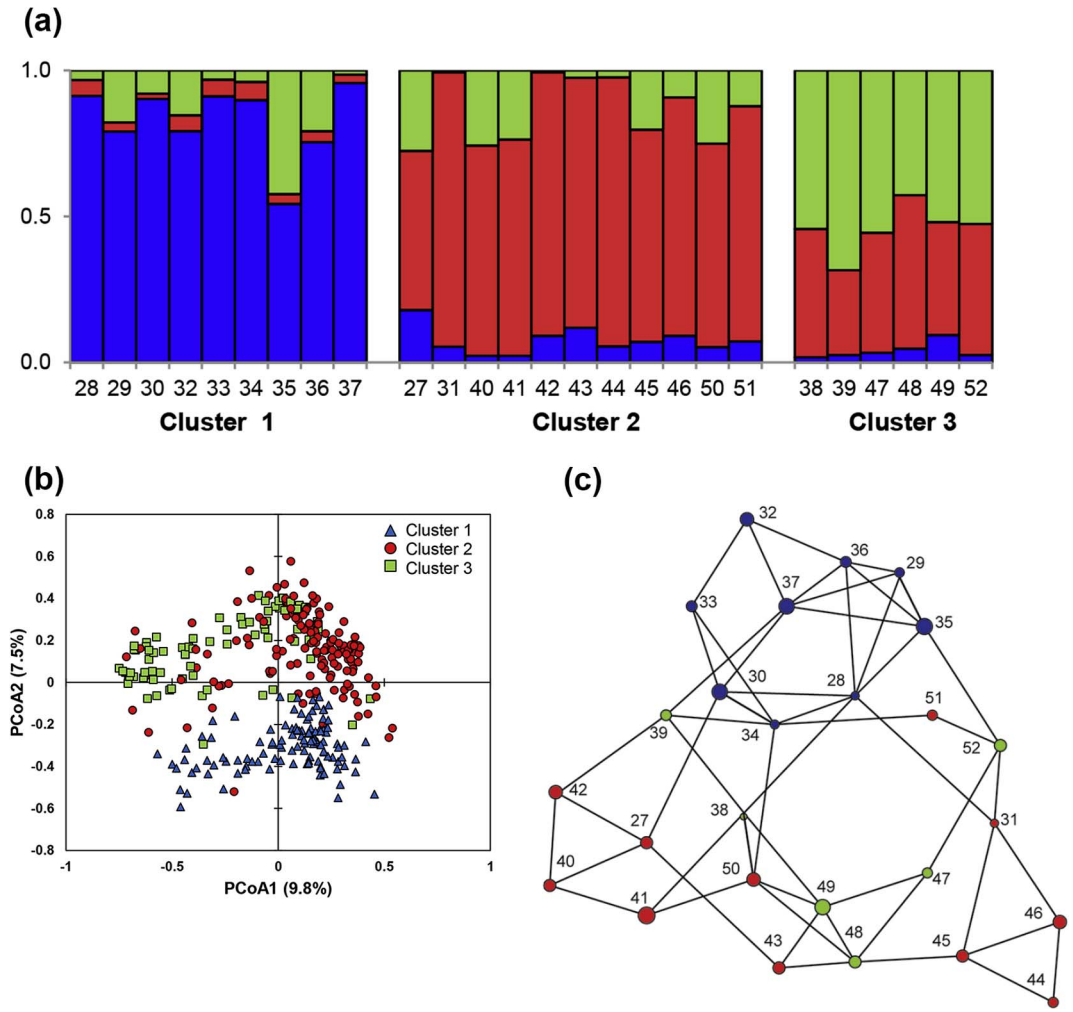

Fig. 3. Population genetic structure analyses for *Sagittaria platyphylla* from the native USA range. (a) Bar plot of mean estimated membership coefficients (q) of each population in $K = 3$ clusters determined by STRUCTURE, (b) Principal coordinate analysis based on Lynch and Milligan's (1994) pairwise comparisons of relatedness (r) among individuals, and (c) PopGraph generated by Genetic Studio showing linkages between populations. Individuals and populations are colored according to their assignment to the genetic clusters determined by STRUCTURE: Cluster 1 (blue), Cluster 2 (red) and Cluster 3 (green). Refer to Table 1 for sampling information.

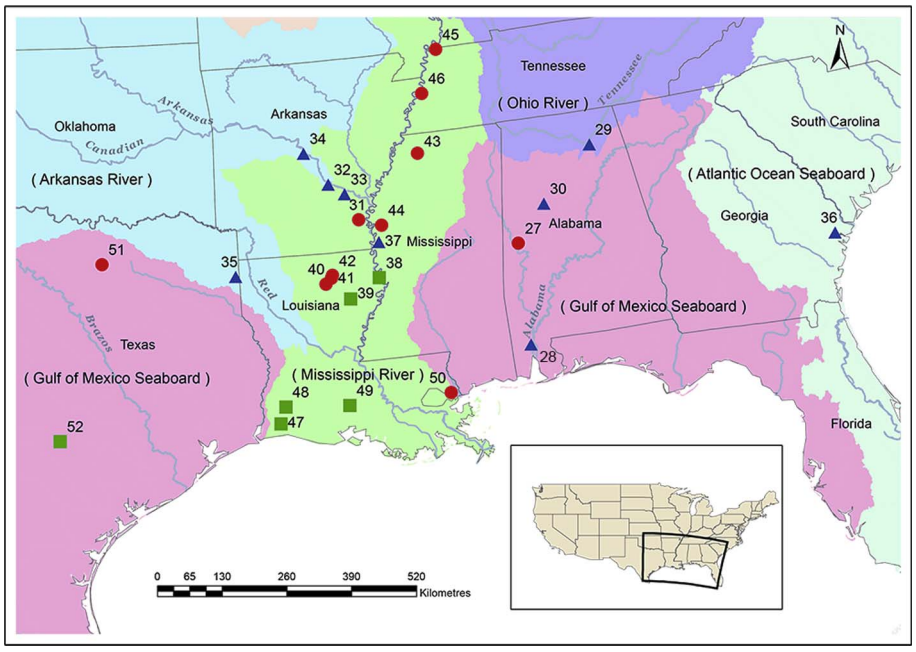

Fig. 4. *Sagittaria platyphylla* population locations sampled in the native USA range. Map colors represent Level II Watershed Regions (provided by the Commission for Environmental Cooperation). Symbol colors correspond to Clusters assigned by STRUCTURE analysis; Cluster 1 (blue), Cluster 2 (red) and Cluster 3 (green). Refer to Table 1 for sampling information.

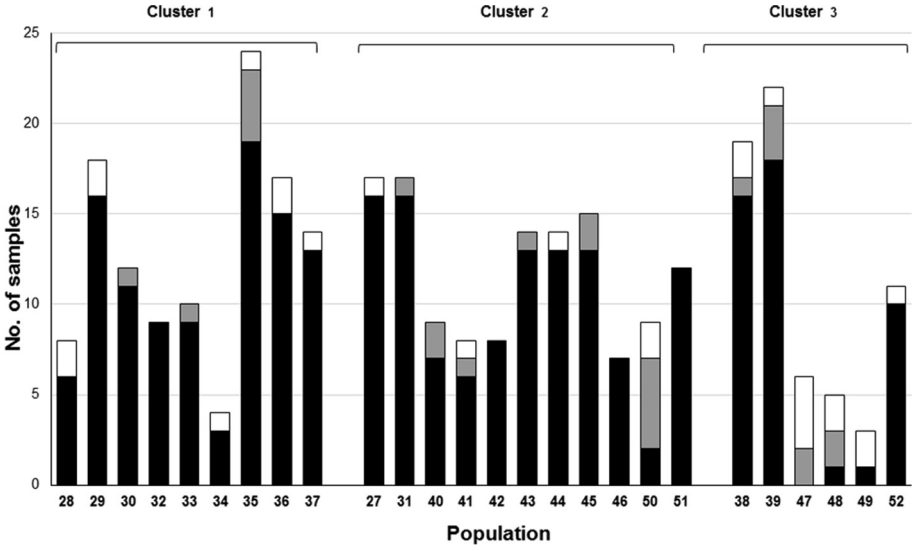

Fig. 5. AFLPOP used to determine the probability of incorrectly assigning USA *Sagittaria platyphylla* samples to their source populations. Assignment to source populations (black), another population (grey) or not allocated (white). Refer to Table 1 for sampling information.

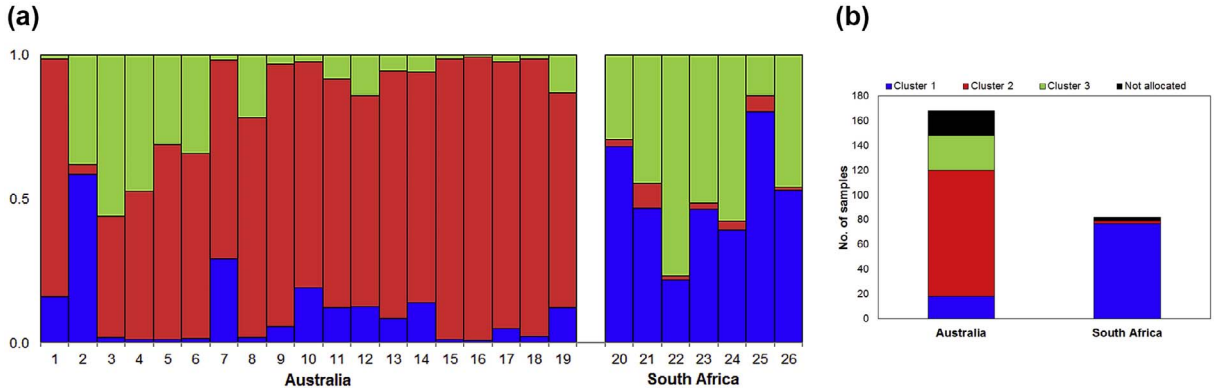

Fig. 6. Assignment of Australian and South African *Sagittaria platyphylla* samples. (a) Populations are color-coded according to their assignment to the genetic clusters determined by STRUCTURE: Cluster 1 (blue), Cluster 2 (red) and Cluster 3 (green), (b) AFLPOP, probability of assignment to source populations generated by STRUCTURE (clusters 1–3) or not allocated (black).

2010). The finding of three genetic clusters primarily associated with expected dispersal patterns along watersheds in the USA allowed for the possible origins of invasive *S. platyphylla* populations to be inferred.

Overall, the STRUCTURE and AFLPOP analyses suggested that most Australian individuals were strongly assigned to Cluster 2 (Fig. 6a, b) which corresponds to populations occurring mostly in upper sections of the Lower Mississippi Region, from Monroe LA to Reelfoot Lake TN (Fig. 4). In contrast, Australian samples from northern Victoria's Barmah Forest (Pops 3–6) were more aligned with Cluster 3 suggesting that a separate introduction from the southern parts of the native distribution such as Louisiana is likely. Evidence from Bayesian assignment and multiple linkages highlighted by PopGraph (see below) point to a third introduction into Melbourne from Cluster 1, potentially from populations beyond the Lower Mississippi Region such as the Arkansas-White-Red Region or the South Atlantic Gulf. Interestingly, a small number of individuals assigned to Cluster 1 also occurred in other Australian populations suggesting that Melbourne may have been a source of secondary invasion to other regions such as the irrigation districts of central Victoria (Pops 7 and 10) and possibly to Albany in Western Australia (Pop 1). Relatively few examples of admixed individuals were evident with plants primarily assigned to one of the three clusters, however, some admixed individuals were present at Pops 1, 7, 10 and 12 for Clusters 1 and 2, Pop 2 (Clusters 1 and 3), and the Barmah populations (Pops 3–6; Clusters 2 and 3; Supplementary material Fig. S2). For invasive *S. platyphylla* in South Africa, these data suggest either multiple introductions from two different parts of the native range, or a single large introduction from a diverse gene pool. Unlike many Australian samples which assigned to Cluster 2, South African samples were assigned to either Cluster 1 or Cluster 3 (Fig. 6a, b; Supplementary material Fig. S3), eliminating the northern parts of the Lower Mississippi Region as being likely source populations.

PopGraph revealed a complex topology of four sub-groups: the USA, two Australian sub-groups and South Africa (Fig. 7). Australian populations from Barmah and Ovens sites in northern Victoria formed a distinct subgroup which was connected to the remaining Australian populations through Pop 9. Several populations in the other Australian sub-group were linked to various USA populations distributed across several watersheds (e.g. Australian Pops 9, 14, 12 and 19 and USA Pops 44, 52, 49 and 47) while other populations (e.g. Pop 13, 7 and 2) were linked to multiple sites within the USA, some of which were in different watersheds. South African populations formed a discrete, highly connected cluster with connections to both USA and Australian populations through both single and multiple linkages. For example, Pops 20 and 25 were both linked with USA Pop 36, Pop 22 was linked to this latter population as well as to Pop 29 while Pop 21 connected with Pop 37 suggesting a greater linkage through Cluster 1 identified from the STRUCTURE analysis. Linkages between South Africa and Australia were through Pop 26 and 11 as well as Pops 22 and 2. Given the two PopGraph linkages between Australia and South Africa in Fig. 7 and the similar assignment of Melbourne and South African individuals (Supplementary material Fig. S3) it is also possible that material has also been exchanged between the two countries although the direction of this exchange cannot be determined.

3.4. Prospects for biological control

Several authors have suggested that genetically diverse, sexually reproducing invasive plant species may require a diverse array of biocontrol agents, while less genetically diverse, asexually reproducing species may require fewer agents (Burdon and Marshall, 1981; Nissen et al., 1995). Where invasive species consist of novel genotypes due to hybridization, genetic drift, inbreeding and/or adaptive evolutionary change (Bossdorf et al., 2005; Gaskin et al., 2011), the risk of biocontrol failure increases if highly damaging, pre-adapted natural enemies cannot be matched with the target weed genotype(s) in the invaded range (Gaskin et al., 2011). The initial purpose of this genetic study was to reveal the likelihood for successful biocontrol of *S. platyphylla* in Australia and South Africa based on the degree of genetic variability and divergence between native and invasive populations.

Introduced populations of *S. platyphylla* in South Africa and Australia show similar levels of genetic diversity comparable to native populations, with the majority of this diversity residing within rather than among populations or countries. In addition, Australian and South African populations can be readily assigned to clusters within the USA and connections with potential source populations identified making the selection of genotype-specific natural enemies possible. These findings bode well for biocontrol success particularly in South Africa, but may be complicated by possible hybridization in some Australian populations. *Sagittaria platyphylla* from the Barmah Forest were divergent to other Australian populations and examples of plants identifiable with *Sagittaria macrophylla* Zucc. as well as examples of intermediates between *S. platyphylla* and *S. macrophylla* suggest that interspecific hybridization may have occurred (Adair et al., 2012). Moreover, it is likely that this has occurred *in situ* in Australia since the natural range for *S. macrophylla* (southern Mexico) is highly disjunct from the native range of *S. platyphylla* (Keener, 2005). This hybridization may influence biocontrol effectiveness if a candidate agent is highly specialized to species-specific genotypes such as biotrophic plant pathogens and eriophyid mites, where genotype-specific pathogenicity may mean that a novel genotype is less susceptible to attack. As no pathogens or eriophyid mites with potential for biological control of *S. platyphylla* were identified in natural enemy surveys (Kwong et al., 2014), the use of broadly-specialized insect agents may overcome any genetic issues associated with the Australia Barmah populations. Of further consideration is that South African and Australian populations were founded by different source populations and consequently, collections of biocontrol agents from the native range should be undertaken separately for each country. The common practice of swapping biocontrol agent cultures between collaborating countries would need to be evaluated for this species, depending on the degree of host specialization of the candidate biocontrol agents. However, the evolution of host-specific natural enemy biotypes is more likely for host species with wide, disjunct geographic native distributions (Goolsby et al.,

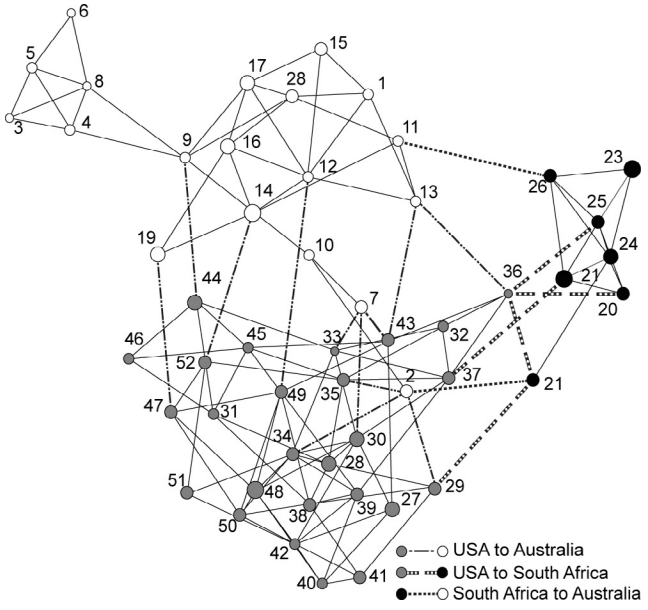

Fig. 7. PopGraph generated by Genetic Studio showing linkages between populations of *Sagittaria platyphylla* from native USA (grey nodes) and invasive Australian (open nodes) and South African (black nodes) populations. Node size indicates within population genetic variance and lines connecting nodes are retained edges indicating genetic covariance. Refer to Table 1 and Fig. 1 for sampling information.

2006), unlike *S. platyphylla*. In addition, no evidence of spatially-specific *S. platyphylla* biotypes in the native range have been found suggesting that natural enemies are likely to be ubiquitous, but this requires confirmation by herbivore transfer experiments (e.g. Goolsby et al., (2006)). At the very least we suggest that potential biocontrol agents be sourced from regions of the closest genetic match to invasive populations and that their performance (survival, reproduction and damage to host) be subsequently assessed against the range of genotypes present in the invaded range(s) (Gaskin et al., 2011).

Data accessibility

STRUCTURE input files: Dryad doi: 10.5061/dryad.f7g58.

Author contributions

R.K. and L.B. jointly conceived the study, analyzed the data and prepared the manuscript. R.K. collected plant samples from Australia and the USA, G.M. and J.C. collected samples from South Africa and B.K. confirmed plant specimen identifications. L.B. supervised the DNA extraction, generated the AFLP data matrix and generated the PopGraph and STRUCTURE analyses. R.K. conducted the AMOVA and principal coordinate analyses. N. Kerr conducted the TESS analyses. All authors discussed the results and implications and commented on the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocontrol.2017.06.002.

References

Adair, R., Keener, B., Kwong, R., Sagliocco, J., Flower, G., 2012. The biology of Australian weeds 60. *Sagittaria platyphylla* (Engelmann) JG Smith and *Sagittaria calycina* Engelmann. Plant Prot. Q. 27, 47–58.

AWC, 2012. Weeds of National Significance, Sagittaria (*Sagittaria platyphylla*) strategic plan. http://www.weeds.org.au/WoNS/sagittaria/docs/National_Sagittaria_Strategic_Plan_%28final-June_2013%29.pdf (accessed 14.08.14).

Baldwin Jr., J.T., Speese, B.M., 1955. Chromosomes of taxa of the Alismataceae in the range of Gray's manual. Am. J. Bot. 42, 406–411. http://dx.doi.org/10.2307/2438748.

Blundell, M.J., Phongkham, T., Gale, K.R., 2010. A novel enzyme-linked immunosorbent assay for the detection of Wx-B1 null wheat lines using a monoclonal antibody and its application for the detection of marker heterogeneity within commercial cultivars. J. Cereal Sci. 52, 187–191. http://dx.doi.org/10.1016/j.jcs.2010.05.002.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E., Prati, D., 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144, 1–11. http://dx.doi.org/10.1007/s00442-005-0070-z.

Broadhurst, L., Chong, C., 2011. Examining clonal propagation of the aquatic weed Sagittaria platyphylla. RIRDC Publication No. 11/020, Project No. AWRC 08–65.

Burdon, J.J., Marshall, D.R., 1981. Biological control and the reproductive mode of weeds. J. Appl. Ecol. 18, 649–658. http://dx.doi.org/10.2307/2402423.

Charudattan, R., Riches, C., 2001. Are we on top of aquatic weeds? Weed problems, control options and challenges. In: The world's worst weeds, Hilton Brighton Metropole Hotel, UK, 12 November 2001. British Crop Protection Council, pp 43–68.

Chen, C., Durand, E., Forbes, F., FranÇOis, O., 2007. Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Mol. Ecol. Notes 7, 747–756. http://dx.doi.org/10.1111/j.1471-8286.2007.01769.x.

Cuda, J.P., Christ, L.R., Manrique, V., Overholt, W.A., Wheeler, G.S., Williams, D.A., 2012. Role of molecular genetics in identifying 'fine tuned' natural enemies of the invasive Brazilian peppertree, *Schinus terebinthifolius*: a review. Biocontrol 57, 227–233. http://dx.doi.org/10.1007/s10526-011-9418-y.

Duchesne, P., Bernatchez, L., 2002. Aflpop: a computer program for simulated and real population allocation, based on AFLP data. Mol. Ecol. Notes 2, 380–383. http://dx.doi.org/10.1046/j.1471-8286.2002.00251.x.

Dyer, R.J., Nason, J.D., 2004. Population graphs: the graph theoretic shape of genetic structure. Mol. Ecol. 13, 1713–1727. http://dx.doi.org/10.1111/j.1365-294X.2004.02177.x.

Earl, D., vonHoldt, B., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet Resour. 4, 359–361. http://dx.doi.org/10.1007/s12686-011-9548-7.

Estoup, A., Guillemaud, T., 2010. Reconstructing routes of invasion using genetic data: why, how and so what? Mol. Ecol. 19, 4113–4130. http://dx.doi.org/10.1111/j.1365-294X.2010.04773.x.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620. http://dx.doi.org/10.1111/j.1365-294X.2005.02553.x.

Evans, K.J., Jones, M.K., Roush, R.T., 2005. Susceptibility of invasive taxa of European blackberry to rust disease caused by the uredinial stage of *Phragmidium violaceum* under field conditions in Australia. Plant. Pathol. 54, 275–286. http://dx.doi.org/10.1111/j.1365-3059.2005.01183.x.

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

Gaskin, J.F., et al., 2011. Applying molecular-based approaches to classical biological control of weeds. Biol. Control 58, 1–21. http://dx.doi.org/10.1016/j.biocontrol.2011.03.015.

GenStat, 2013. GenStat for Windows. VSN International, Oxford.

Goolsby, J.A., De Barro, P.J., Makinson, J.R., Pemberton, R.W., Hartley, D.M., Frohlich, D.R., 2006. Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. Mol. Ecol. 15, 287–297. http://dx.doi.org/10.1111/j.1365-294X.2005.02788.x.

Guillot, G., 2009. On the inference of spatial structure from population genetics data. Bioinformatics 25, 1796–1801. http://dx.doi.org/10.1093/bioinformatics/btp267.

Hauber, D.P., Lege, L., 1999. A survey of allozymic variation among three members of the *Sagittaria graminea* complex (Alismataceae) from the southeastern United States. J. Torrey Bot. Soc. 126, 181–187. http://dx.doi.org/10.2307/2997273.

Hufbauer, R.A., 2004. Population genetics of invasions: can we link neutral markers to management? Weed Technol. 18, 1522–1527. http://dx.doi.org/10.1614/0890-037x(2004)018[1522:pgoicw]2.0.co;2.

Hussner, A., 2012. Alien aquatic plant species in European countries. Weed Res. 52, 297–306. http://dx.doi.org/10.1111/j.1365-3180.2012.00926.x.

Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801–1806. http://dx.doi.org/10.1093/bioinformatics/btm233.

Julien, M.H., Griffiths, M.W., 1998. Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds, 4 Ed. CABI, Wallingford, UK.

Keener, B.R., 2005. Molecular systematics and revision of the aquatic monocot genus Sagittaria (Alismataceae). PhD dissertation University of Alabama, Tuscaloosa, Alabama.

Kwong, R., Sagliocco, J.-L., Harms, N., Shearer, J.F., Keener, B., Green, P., 2014. Prospects for the biological control of delta arrowhead (*Sagittaria platyphylla*), an invasive aquatic species in Australia. In: Impson F.A.C., Kleinjan C.A., Hoffmann J.H. (Eds.), XIV international symposium on biological control of weeds, Kruger National Park, South Africa, 2-7 March 2014; 2014, pp 53–67.

Kwong, R.M., Sagliocco, J.L., Harms, N.E., Butler, K.L., Green, P.T., Martin, G.D., 2017. Biogeographical comparison of the emergent macrophyte, *Sagittaria platyphylla* in its native and introduced ranges. Aquat. Bot. 141, 1–9. http://dx.doi.org/10.1016/j.aquabot.2017.05.001.

Lynch, M., Milligan, B.G., 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3, 91–99. http://dx.doi.org/10.1111/j.1365-294X.1994.tb00109.x.

McFadyen, R.E., 1999. Successes in biological control of weeds. In: Spencer, N.R. (Ed.), X international symposium on biological control of weeds. Montana State University, Bozeman, Montana, USA, pp. 3–14.

Müller-Schärer, H., Schaffner, U., Steinger, T., 2004. Evolution in invasive plants: implications for biological control. Trends Ecol. Evol. 19, 417–422. http://dx.doi.org/

10.1016/j.tree.2004.05.010.
Nissen, S.J., Masters, R.A., Lee, D.J., Rowe, M.L., 1995. DNA-based marker systems to determine genetic diversity of weedy species and their application to biocontrol. Weed Sci. 504–513.
National Library of Australia. Trove. Digitized newspapers (2014) http://trove.nla.gov.au/newspaper/result?q=+Sagittaria. Accessed 1 August.
O'Hanlon, P., Peakall, R., Briese, D., 2000. A review of new PCR-based genetic markers and their utility to weed ecology. Weed Res. 40, 239–254. http://dx.doi.org/10.1046/j.1365-3180.2000.00191.x.
Paynter, Q., Overton, J.M., Hill, R.L., Bellgard, S.E., Dawson, M.I., 2012. Plant traits predict the success of weed biocontrol. J Appl Ecol 49:1140–1148. doi:10.1111/j.1365-2664.2012.02178.xPeakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295. http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x.
Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28, 2537–2539. http://dx.doi.org/10.1093/bioinformatics/bts460.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Sagliocco, J.-L., Ainsworth, N., Adair, R.J., 2007. Arrowhead in Victoria: current control methods and potential for biological control. In: 3rd Victorian Weeds Conference, 'Earth, Wind, Fire and Weeds', Frankston, Victoria, 2007. Weed Society of Victoria, pp 78–79.
Vekemans, X., 2002. AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Genetique et Ecologie, Universite Libre de Bruxelles, Belgium.
Vos, P., et al., 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23, 4407–4414. http://dx.doi.org/10.1093/nar/23.21.4407.
Zhivotovsky, L.A., 1999. Estimating population structure in diploids with multilocus dominant markers. Mol. Ecol. 8, 907–913. http://dx.doi.org/10.1046/j.1365-294x.1999.00620.x.