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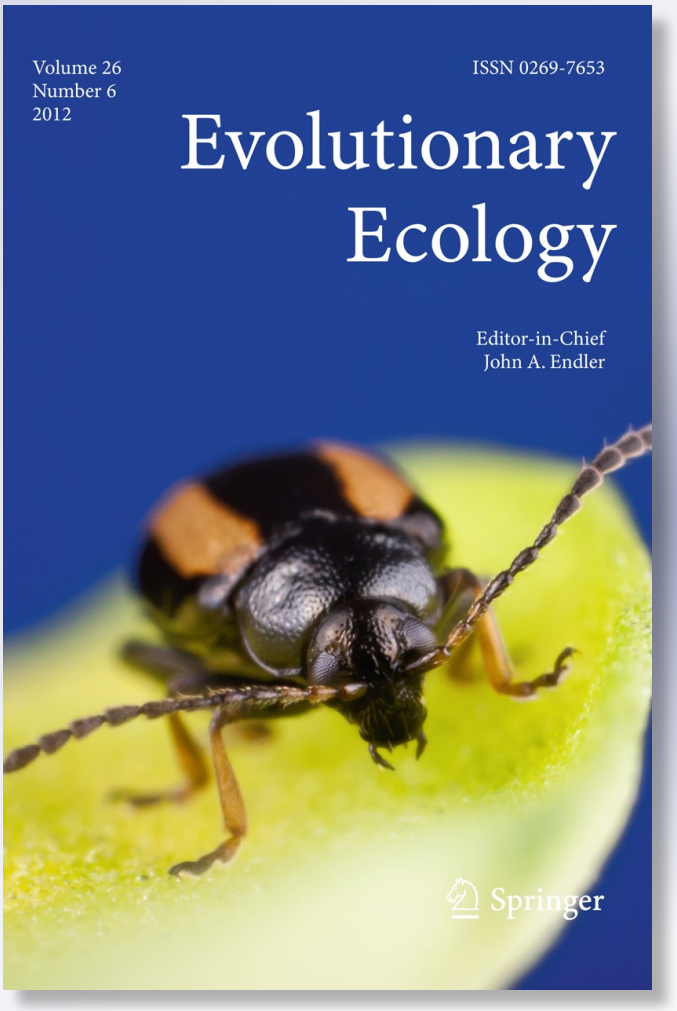
Evolutionary Ecology

ISSN 0269-7653
Volume 26
Number 6

Evol Ecol (2012) 26:1345-1360
DOI 10.1007/s10682-012-9570-6



Springer

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ORIGINAL PAPER

No consistent association between changes in genetic diversity and adaptive responses of Australian acacias in novel ranges

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Received: 11 October 2011 / Accepted: 22 March 2012 / Published online: 5 April 2012

Abstract Common garden studies comparing trait differences of exotic species between native and introduced ranges rarely incorporate an analysis of genetic variation, but simply infer that trait shifts between ranges are genetically determined. We compared four growth-related traits (total biomass, relative growth rate RGR, specific leaf area SLA, and root to shoot ratio R:S) of five invasive Fabaceae species (*Acacia cyclops, A. longifolia, A. melanoxylon, A. saligna, Paraserianthes lophantha*), grown in a common garden experiment using seeds from introduced and native ranges across Australia. Chloroplast microsatellite loci were used to compare genetic diversity of native and introduced populations to determine standing genetic diversity and infer introduction history. We asked whether shifts in traits associated with faster growth due to enemy release in the introduced range were associated with levels of genetic diversity associated with introduction history. We found differences in traits between ranges, although these traits varied among the species. Compared to native-range populations, introduced-range *Acacia longifolia* had greater biomass

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Electronic supplementary material The online version of this article (doi:10.1007/s10682-012-9570-6) contains supplementary material, which is available to authorized users.

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and larger SLA; *A. cyclops* had greater RGR; and *A. melanoxylon* displayed lower R:S. Genetic diversity in the introduced range was lower for one of those species, *A. longifolia*, and two others that did not show differences in traits, *A. saligna and P. lophantha*. Diversity was higher in the introduced range for *A. melanoxylon* and did not differ among ranges for *A. cyclops*. These patterns of genetic diversity suggest that a genetic bottleneck may have occurred following the introduction of *A. longifolia, A. saligna* and *P. lophantha*. In contrast greater or comparable genetic diversity in the introduced range for *A. melanoxylon* and *A. cyclops* suggests introductions from multiple sources. This study has shown that a reduction in genetic diversity in the introduced range is not necessarily associated with a reduced capacity for adaptive responses or invasion potential in the novel range.

Keywords *Acacia* · Adaptive capacity · Common garden · Genetic variation · Invasive plants · Relative growth rate · Specific leaf area

Introduction

The characteristics of successful invaders and the role of genetic variation in promoting invasiveness are central themes in invasion biology. Variation in some important ecological traits has been consistently associated with invasion success (Sakai et al. 2001; Hamilton et al. 2005; Rejmánek et al. 2005; Leishman et al. 2007; Schlaepfer et al. 2010), with successful invaders being either pre-adapted to a novel environment or capable of phenotypic or genetic adaptation to novel conditions, including freedom from enemies. Recent reviews (e.g. Prentis et al. 2008) have highlighted the potential for rapid adaptive evolution in plants introduced to novel environments, and an increased understanding of the mechanisms underlying adaptation will be essential for understanding species' adaptive capacity under climate change.

A range of studies have examined evidence for shifts in traits associated with faster growth strategies and higher fecundity in introduced populations, using both field studies and common garden experiments. Many studies have found evidence for greater biomass or reproductive output in individuals from the introduced range (e.g. Siemann and Rogers 2001; Lleger & Rice 2003; Maron et al. 2004; Caño et al. 2008; Barney et al. 2009), while other studies have not (e.g. Willis et al. 2000; van Kleunen and Schmid 2003; Cripps et al. 2009). Teasing apart the influence of heritable, genetically based variation in introduced populations from environmentally induced variation requires analysis of trait variation of individuals from native and introduced ranges under common garden conditions. Although common garden experiments are a particularly useful technique for disentangling the contribution of genetic and environmental factors in the success of exotic species in their introduced range (Colautti et al. 2009; Moloney et al. 2009), few studies have explicitly combined trait comparisons with genetic analysis (but see Handley et al. 2008; Chun et al. 2011). Comparison of genetic structure and variation among populations from the original and introduced ranges provides insight into the introduction pathways of the exotic species and can potentially reveal genetic mechanisms underlying phenotypic variation expressed in the introduced range that contributes to invasive success.

The importance of genetic diversity for the invasion success of species introduced to novel environments remains unclear. While some invasions are characterized by high or even increased levels of genetic diversity (e.g. Lavergne and Molofsky 2007; Marrs et al. 2008), many are characterized by reduced genetic variation due to bottlenecks associated with founder events (Dlugosch and Parker 2008), and for some species different invasive

ranges can exhibit high or low diversity (Kang et al. 2007). Genetic bottlenecks have traditionally been considered an impediment to invasion success, decreasing adaptive responses to new environmental conditions (Nei et al. 1975) and adversely affecting variation in ecologically important traits. However, empirical studies have shown that low levels of standing genetic variability do not necessarily limit invasion success and furthermore that many invasive species do not experience genetic bottlenecks (Naciri-Graven and Goudet 2003; Le Roux et al. 2007; Prentis et al. 2008, 2009).

Genetic diversity of populations in novel ranges is likely to be strongly linked to the introduction pathway and history of the species. Multiple introductions have been proposed as a potentially important mechanism by which founder effects in the introduced range can be overcome by increasing both population size and population growth rate (Ellstrand and Schierenbeck 2000). Multiple independent introductions can also generate diversity via mechanisms such as intra- or interspecific hybridization (Novak et al. 1993; Maron et al. 2004; Genton et al. 2005; Wilson et al. 2009; Simberloff 2009; Dormontt et al. 2011). Multiple introductions are relatively common in invasive species (Wilson et al. 2009; Dormontt et al. 2011) and have the potential to introduce large amounts of genetic variation as well as give rise to genetic novelty (Dlugosch and Parker 2007; Prentis et al. 2008). Understanding the links between invasion history (i.e. propagule pressure, number of introduction events, introduction sources), standing genetic variation and invasion success is important for understanding the evolutionary outcomes of invasions and how to incorporate them into management strategies for invasive species. However, the relationships between these parameters are not straightforward and can be obscured by numerous processes such as the genetic structure of native range populations, and number of sources versus number of introductions (see Le Roux et al. 2011; Fig. 1 for details).

Acacia is a large, widespread genus containing over 1380 species, the majority of which originate from Australia (Maslin et al. 2003). Globally, many *Acacia* species have been introduced into areas outside their natural distribution for a variety of reasons including environmental services, ornamental and forestry purposes (Musil 1993; Witkowski 1994; Yelenik et al. 2004; Kull et al. 2008; Richardson and Rejmànek 2011), with approximately 20 of these species recognised as invasive (Richardson and Rejmànek 2011). As with many plant introductions, human-mediated redistribution of some acacias around the world has resulted in significant ecological impacts within new environments (Le Maitre et al. 2011). In South Africa for example, Australian native species such as *Acacia saligna* and *Acacia cyclops* displace native vegetation (Richardson et al. 1989; Gaertner et al. 2009), reduce native species regeneration and colonization (Musil 1993; Tassin et al. 2009), and facilitate secondary invasions (Yelenik et al. 2004). Within Australia, the anthropogenic movement of acacias into areas outside their original range has caused significant ecological disruption (Costello et al. 2000; Emms et al. 2005). For example, it is estimated that 76 % of native plant species disappeared within 20 years following the introduction and subsequent invasion of *Acacia sophorae* (syn. *Acacia longifolia* subsp. *sophorae*) into coastal grasslands of south-eastern Australia (Costello et al. 2000).

In this study we examine trait and genetic variation between native and introduced ranges of five closely related woody legumes (four *Acacia* species and the closely-related *Paraserianthes lophantha*). All five species are known to be invasive outside their native range, both within Australia and on other continents. Growth-related trait data were derived from a common garden experiment. Using diversity at chloroplast microsatellite loci, we compared genetic diversity of native and invasive range populations to infer introduction history. These approaches allow us to examine how the standing genetic variation introduced during invasions is associated with the adaptive potential of invasive

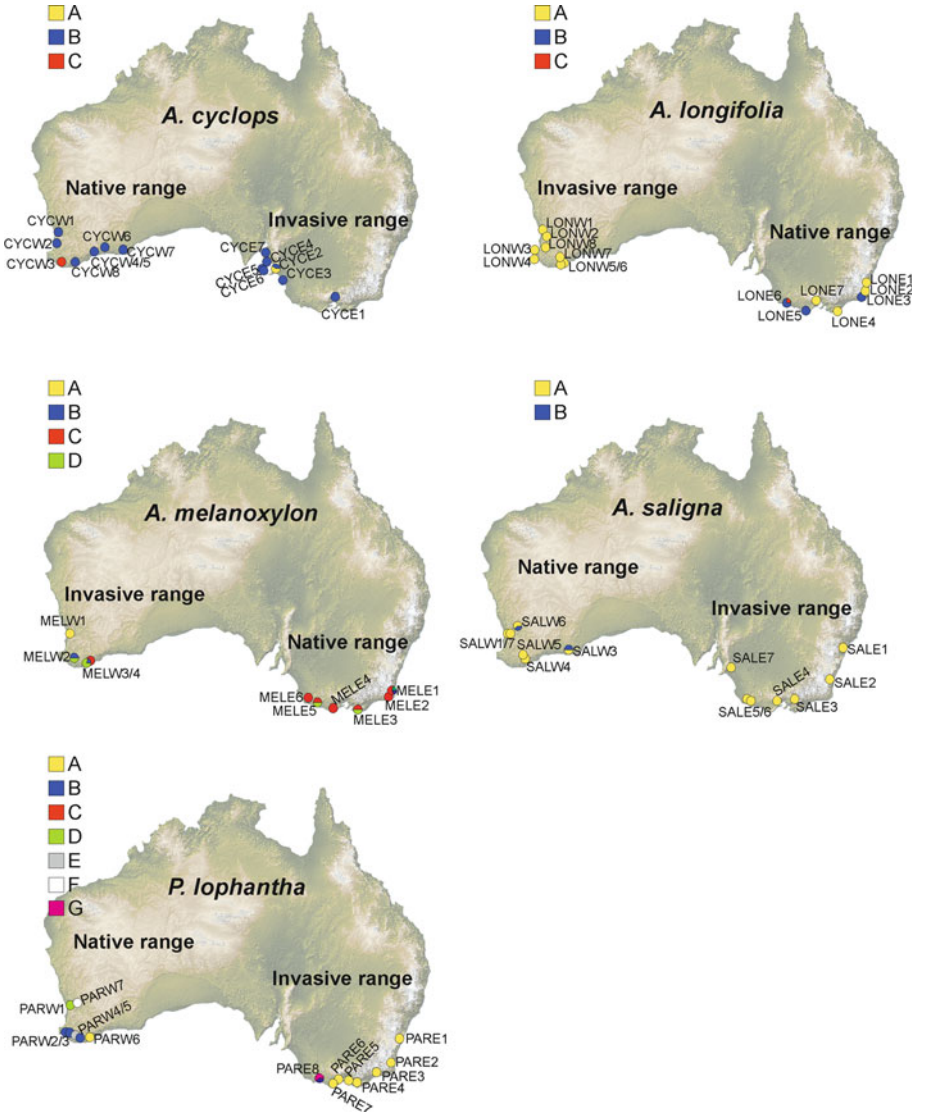

Fig. 1 Maps illustrating chloroplast haplotype distributions and frequencies of populations sampled from the introduced and native ranges of five species of Fabaceae

populations across a phylogenetically controlled group. We hypothesize: (1) that introduced populations possess traits indicating a shift towards faster growth strategies and greater total biomass through higher relative growth rate, specific leaf area and lower root to shoot ratio; (2) that genetic variation will be lower in introduced populations due to genetic bottlenecks arising from small founding populations; and (3) that genetic bottlenecks resulting from introduction constrain invasive potential such that species with reduced genetic diversity will not exhibit shifts towards faster growth strategies in the introduced range.

Methods

Study species

This study focused on five species from the Fabaceae family (sub-family Mimosoidae, genera *Acacia* and *Paraserianthes*). Two of the five species (*A. longifolia* and *A. melanoxylon*) are native to eastern Australia and have become naturalized or invasive in western Australia, while the other three species (*A. cyclops*, *A. saligna, P. lophantha)* are native to western Australia and have become naturalized or invasive in eastern Australia. The two areas are separated by the vast Nullabor Plain, which acts as an effective barrier to natural dispersal. All species have been introduced to the opposite side of the continent to their native range as a result of human activities (e.g. horticultural and forestry purposes). All study species have been declared as naturalized within their introduced ranges in Australia (Randall 2007), although they vary in their abundance and extent. Differences in abundance and distribution between the study species in their introduced ranges in Australia may be partly attributable to differences in residence time and the number of introduction attempts (Table 1).

The taxonomy of *A. longifolia* and *A. saligna* in particular warrant specific mention. Firstly, *A. longifolia* has two subspecies, subsp. *longifolia* and subsp. *sophorae*, which are capable of hybridization, with intermediate forms between the subspecies occurring. Both subspecies have become naturalized in western Australia (Harden 2002; Muyt 2001; Weber 2003) and we therefore sampled both sub-species in each range. For *A. saligna*, four main variants are recognised in its native range, although only three genetically distinct groups have been identified (George et al. 2005; Millar et al. 2011). There is still discrepancy between

Table 1 Distribution, abundance and number of populations sampled for study species across their native and introduced ranges

Native Range	Species	Abundance in native range	Abundance in introduced range	No. populations sampled, native range	No. populations sampled, introduced range
Eastern Australia	*A. longifolia*	Widespread[#]	Naturalized[#], troublesome weed[^], forms dense stands[§]	8	8
Eastern Australia	*A. melanoxylon*	Widespread, often common[#]	Naturalized and spreading[*]	7	4
Western Australia	*A. cyclops*	Widespread[*]	Naturalized[◇], infrequent[§]	8	7
Western Australia	*A. saligna*	Widespread, often locally abundant[*]	Naturalized, often weedy[*], aggressive[#]	7	7
Western Australia	*P. lophantha*	Widely distributed but uncommon[§]	Widely naturalized but not common[#], locally abundant[§]	7	9

Herbarium records (Australia's Virtual Herbarium http://www.chah.gov.au/avh/) and published literature were used to assess relative abundance across ranges for each species

[#] Botanic Gardens Trust 2010, [*] Maslin and McDonald 2004, [^] Moore and Wheeler 2008, [§] Personal observations, [◇] Virtue and Melland 2003

identification of these variants and the distinct differences between phenotypes and their corresponding genotypes are not yet resolved (World Wide Wattle 2010; http://www.worldwidewattle.com/). Because it is not possible to accurately distinguish between variants, we did not target our sampling to one type and were likely to have covered all variants.

The study was conducted across the study species' native and introduced ranges in south-eastern Australia and south-western Australia. The sampling range in the south-western region of Australia experiences a Mediterranean climate, with distinct rainy periods in the winter, and hot dry summers. The south-eastern Australian range is predominantly temperate, with warm summers and mild winters. We sampled populations in eastern Australia, from Sydney (33°46.13′S, 151°16.07′E) to Yorke Peninsula (34°30.1′S, 137°39.2′E) and in western Australia, from Perth (31°51.09′ S, 115°50.26′ E) to Esperance (33°51.02′ S, 121°53.10′ E), ensuring that as much of each species' range (native and introduced) was sampled as possible. Sampling was conducted during optimum fruiting production over the summer of 2008–2009.

Sampling procedure

We aimed to collect a representative sample of each species within each range, as recommended for common garden experiments (Moloney et al. 2009). Where possible, we collected seed samples from a minimum of five populations within both the native and introduced ranges of each species, although the number sampled varied among species (range 4–9) due to availability of sites where sufficient individuals were fruiting (Table 1). *Acacia melanoxylon* in particular has a limited distribution in its introduced range in south-western Australia and thus only four populations were sampled. At each site, we sampled at least three, but usually five individuals that were producing mature pods and seeds. Leaf samples were collected from each tree and were stored in paper bags containing silica gel for desiccation prior to DNA extraction.

Common garden experiment

A common garden experiment was conducted under standard glasshouse growth conditions using seeds collected from introduced and native range populations for all five study species. Twenty seeds from each tree within each population for both ranges of the five species were selected for germination, based on weight. It is generally recommended that common garden experiments account for maternal effects (Moloney et al. 2009). Seed size is a commonly measured maternal effect that affects seedling size, survival and growth (e.g. El-Keblawy and Lovett-Doust 1998). Ideally, the F1 generation (i.e. offspring from field-collected seeds) would be used in common garden experiments in order to account for maternal effects. However, owing to the long lived nature of our study species (i.e. 3–5 years to reproductive maturity), this was not a feasible option. We therefore used only seeds that were within one standard deviation of the measured mean seed mass for that species in order to minimise any maternal effects. Seeds were pre-treated by sterilizing them in a 6 % bleach solution for 5 min. Germination was then promoted by immersing seeds in boiling water for 1 min. The 20 seeds per tree were placed on filter paper in petri dishes which also contained a small piece of saturated sponge that acted as a water reservoir. Petri dishes were then sealed. Seeds were germinated in growth cabinets (LABEC, Marrickville, NSW, Australia) set at 25° C with a photoperiod of 12 h.

Following emergence, seedlings were transferred to pots (9 cm diameter, 13.5 cm deep) containing 0.6 L of soil mixture consisting of organic garden mix and coarse river sand in a

ratio of 3:2. Pots were lined with newspaper to prevent soil loss. Seedlings from the four *Acacia* species were transplanted at the stage of cotyledon emergence. The *P. lophantha* seedlings were planted at the stage of full emergence. Whenever possible, 10 seedlings per population per range were planted. All seedlings of each of the five species were planted within 24 h of each other to ensure equal length of growing time.

Ideally common garden experiments should be conducted in multiple gardens within both ranges (Moloney et al. 2009); however, this approach is not feasible with multiple species whose ranges do not entirely overlap. Instead we conducted a single common garden experiment using standard glasshouse growth conditions, typical of the native and introduced ranges of all five species. Plants were grown for a 12 week period in a glasshouse with a temperature range of 19–25 °C. To avoid bias due to variation in growing conditions within the glasshouse, pots were randomly moved to new positions fortnightly. All pots were mist watered for two minutes three times daily. To counteract the nutrient loss resulting from this daily watering, 3.5 ± 0.2 g of slow release native plant fertilizer (23 N:2P:17 K, J.R. Somplot Company, Lathrop, California, United States) was added to each pot at the beginning of the experiment.

After the 12 week growth period, all plant components were washed free of soil before being oven-dried at 60 °C for 72 h and weighed. We then measured total biomass, relative growth rate RGR, specific leaf area SLA and root:shoot ratio R:S for each individual. RGR was calculated based on initial seed mass, using the formula $RGR = (\log_e P_M - \log_e S_M)/T$, where P_M is plant mass after growth period (T), being 84 days (12 weeks) and S_M is seed mass. SLA was calculated as leaf area (incorporating leaf blade and petiole) per unit leaf mass. Leaf area was measured using a LI-3100C Area Meter (LI-COR, Lincoln, NE, USA) before being oven-dried, and weighed to obtain dry mass. All weight measurements were made using a Mettler Toledo B–S four decimal place electronic balance.

Genetic diversity analysis

DNA was extracted from silica-dried leaf material from between one and five individuals from the majority of the populations collected for the common garden experiment, along with 15 and 17 samples of *A. cyclops* and *A. saligna*, respectively, which were collected from additional populations in Australia (see Table 1 in supplementary material for full population details). Extractions were outsourced to the Australian Genome Research Facility (AGRF)—Adelaide node, and carried out on the Machery Nagal Plant II system using the PL2/PL3 buffer system.

Ten chloroplast microsatellite primer pairs developed by Weising and Gardner (1999) were screened for one individual for successful amplification from five different native populations of each species. This representative subset was chosen for this locus selection step, as it was assumed that most genetic diversity will reside between rather than within populations. Each PCR reaction (10 μL total volume) contained ~20 ng of template DNA, 1× reaction buffer (supplied with DNA polymerase), 0.2 mM each dNTP, 2.5 mM $MgCl_2$, 0.5 μM of each forward and reverse primer, and 1 U IMMOLASE™ DNA polymerase (Bioline). PCR reactions were carried out with an initial denaturation step of 94 °C for 5 min, followed by 30 cycles of 94 °C for 20 s, 50 °C for 20 s, 72 °C for 20 s, and a final extension at 72 °C for 30 min. PCR products were run on agarose gels stained with ethidium bromide and visualised under UV light. Loci that amplified products of expected size were then used to re-run the PCR reactions using forward primers labelled with florescent dyes (either VIC, FAM, NED or PET). These products were then separated by AGRF using the ABI 3730 DNA analyzer (Applied Biosystems, CA, USA). The GeneScan™–500 LIZ®

size standard was used. Fragments were scored using Genemapper® Software v4.0 (Applied Biosystems, CA, USA). Polymorphic loci were identified for each species (Table 2) and used to screen the remainder of the samples following the method described above.

Statistical analyses

Common garden experiment

The five species were analysed separately as we were interested solely in intraspecific differences in traits between native and introduced ranges. We used a nested ANOVA with populations nested with ranges. Nesting was considered appropriate as individual plants within populations were not independent. Range was treated as a fixed effect and population as a random effect. Species' traits (total biomass, SLA, RGR and R:S) were $\log_{10}$ transformed where necessary to fulfil the assumptions of normality and homogeneity of variances. All analyses were conducted using SPSS version 18.0.0 (IBM SPSS Statistics, 2009).

Genetic diversity analysis

The number of haplotypes in each range for each species was summed from genotype data. GenAlEx v6.4 (Peakall and Smouse 2006) was used to calculate Shannon's index of diversity (Shannon 1948) for individual populations, and the native and invasive ranges as a whole, using the following equation: $H = -\sum p_i \ln(p_i)$ where p_i is the proportion of the ith haplotype in the population. Where more than one locus was scored, this value was averaged over all loci to give a per population estimate. The presence and absence of particular haplotypes in each of the native and invasive populations were recorded.

Results

Common garden experiment

Total biomass

Total biomass of *A. longifolia* was significantly larger (28 %) for seedlings grown from the introduced compared with native range populations (Table 3; Fig. 2a). For *A. melanoxylon*

Table 2 Population and loci information for samples used in the genetic diversity analysis

Species	Native		Introduced		Polymorphic cpSSR loci
	n_p	n_i	n_p	n_i	
A. longifolia	7	2-5	8	2-5	ccmp5, ccmp8
A. melanoxylon	7	1-5	4	2-5	ccmp2, ccmp5, ccmp7
A. cyclops	8	1-5	7	1-5	ccmp5
A. saligna	6	1-5	7	2-5	ccmp7
P. lophantha	7	1-5	8	2-5	ccmp5, ccmp7

Number of populations (n_p) and number of individuals sampled from each population (n_i) is given for each of the native and introduced ranges. Polymorphic chloroplast microsatellites (from those described by Weising and Gardner 1999) are also indicated

Table 3 Summary of nested ANOVA results for total biomass, SLA, RGR and root:shoot ratio comparisons of seedlings grown from seed of native and introduced range populations in a common garden experiment

	Species	Source of variation	*df*	Mean square	F	*P*
Total biomass (g)	*A. longifolia* ($\log_{10}$)	Range	1	0.557	7.799	**0.013**
		Population (Range)	12	0.071	0.976	0.477
	A. melanoxylon	Range	1	0.323	0.903	0.359
	($\log_{10}$)	Population (Range)	8	0.611	4.167	**0.001**
	A. cyclops ($\log_{10}$)	Range	1	0.075	0.545	0.470
		Population (Range)	9	0.161	1.618	0.126
	A. saligna	Range	1	0.012	0.069	0.800
		Population (Range)	8	0.170	1.449	0.193
	P. lophantha	Range	1	0.050	0.244	0.627
		Population (Range)	9	0.259	1.718	0.131
SLA ($mm^2\ mg^{-1}$)	*A. longifolia*	Range	1	77.625	5.934	**0.029**
		Population (Range)	12	14.202	2.075	**0.025**
	A. melanoxylon	Range	1	0.087	1.639	0.232
	($\log_{10}$)	Population (Range)	8	0.108	15.747	**<0.001**
	A. cyclops	Range	1	0.111	0.007	0.936
		Population (Range)	9	17.320	1.108	0.368
	A. saligna ($\log_{10}$)	Range	1	0.000	0.008	0.933
		Population (Range)	8	0.011	1.904	0.074
	P. lophantha	Range	1	0.038	0.001	0.971
		Population (Range)	9	31.128	1.227	0.319
RGR (mg/day)	*A. longifolia*	Range	1	0.000	0.148	0.707
		Population (Range)	11	0.001	12.815	**<0.001**
	A. melanoxylon	Range	1	0.001	4.526	0.053
		Population (Range)	8	0.000	3.562	**0.002**
	A. cyclops	Range	1	0.001	17.931	**<0.001**
		Population (Range)	9	0.000	0.745	0.667
	A. saligna	Range	1	0.000	0.592	0.464
		Population (Range)	8	0.000	2.503	**0.019**
	P. lophantha	Range	1	0.001	7.883	**0.009**
		Population (Range)	9	0.000	0.891	0.545
R:S	*A. longifolia* ($\log_{10}$)	Range	1	0.209	2.938	0.108
		Population (Range)	12	0.077	2.118	**0.021**
	A. melanoxylon	Range	1	0.261	7.466	**0.014**
	($\log_{10}$)	Population (Range)	8	0.051	2.318	**0.031**
	A. cyclops ($\log_{10}$)	Range	1	0.004	0.060	0.809
		Population (Range)	9	0.064	1.165	0.331
	A. saligna ($\log_{10}$)	Range	1	0.007	0.929	0.363
		Population (Range)	8	0.007	5.361	**<0.001**
	P. lophantha	Range	1	0.000	0.016	0.902
		Population (Range)	9	0.015	4.321	**0.001**

$\log_{10}$ transformations shown for each species (when required). Significant *P* values are highlighted in bold

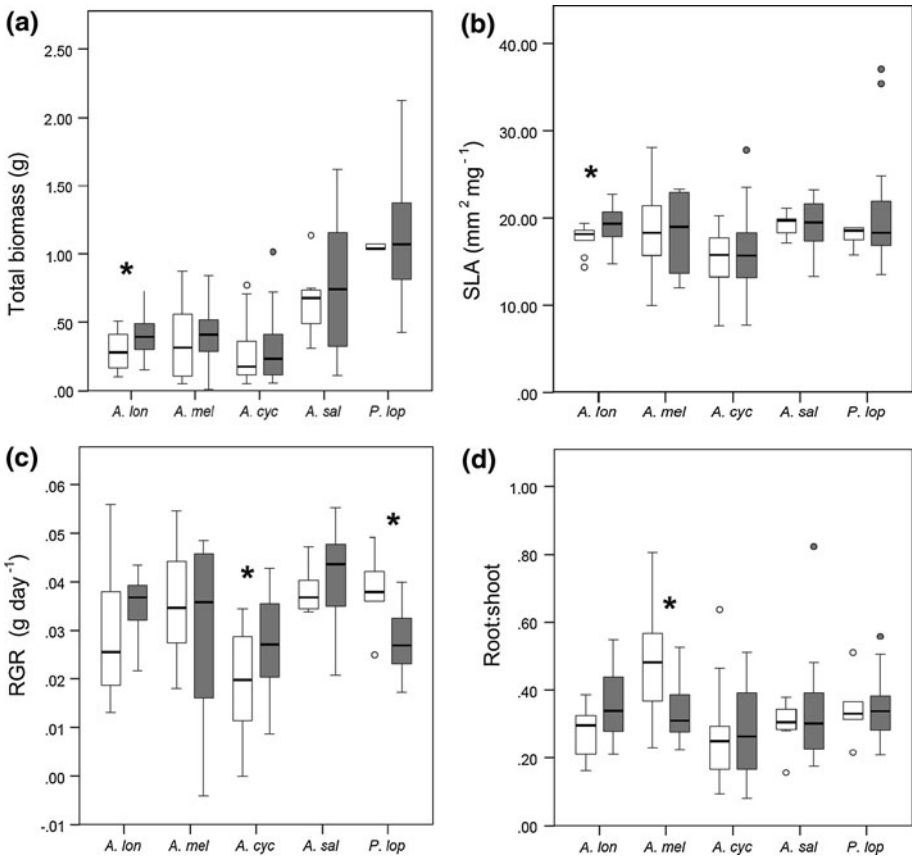

Fig. 2 Box and whisker plots displaying traits of seedlings grown from seed collected from populations within the native (*white plot*) and introduced (*grey plot*) ranges of five species of Fabaceae within Australia. **a** Total biomass; b) specific leaf area SLA; c) relative growth rate RGR and d) root:shoot ratio R:S. Plots are based on median values, whiskers represent lower and upper quartiles, outliers are indicated by circles. Asterisks indicate a significant difference between ranges

we found no significant difference in biomass between seedlings grown from introduced and native ranges but there were significant differences between populations (Table 3, see also supplementary material 1). For the other three species there were no significant differences in total biomass between introduced and native ranges or between populations (Table 3).

Specific leaf area (SLA)

Results for specific leaf area were consistent with the biomass results. There was a significant difference in SLA of seedlings grown from seed of native compared with introduced range populations for *A. longifolia*, with on average, a 9 % increase in SLA in introduced compared to native populations (Table 3; Fig. 2b). A significant difference between populations nested within ranges was also found, with one population in the native range having a much higher SLA than the other native populations, while in the introduced

range a single population had significantly lower SLA than the others (Table 3; Fig. 2b). For *A. melanoxylon* there was no significant difference between ranges but there were significant differences between populations within ranges (Table 3; Fig. 2b). The other three species showed no differences between ranges or populations (Table 3).

Relative growth rate (RGR)

All five species showed differences in RGR between either populations or ranges (Table 3). *A. longifolia*, *A. melanoxylon* and *A. saligna* had significant differences between populations but not between ranges. In contrast both *A. cyclops* and *P. lophantha* had significant differences in RGR between ranges, although for *A. cyclops* RGR was larger for seedlings grown from seed collected from the introduced range while for *P. lophantha* RGR was larger for seedlings grown from seed collected in the native range (Table 3; Fig. 2c).

Root:shoot ratio (R:S)

Differences in R:S were largely driven by particular populations within their respective ranges, with only *A. melanoxylon* displaying lower R:S in its introduced range (Table 3; Fig. 2d). For three of the remaining species (*A. longifolia, A. saligna*, and *P. lophantha*) significant differences in R:S were found at the population level only, while there were no differences between populations or ranges found for *A. cyclops* (Table 3).

Genetic diversity

For all species apart from *A. melanoxylon*, only one or two chloroplast loci were found to be polymorphic (Table 2). As expected for an introduction bottleneck scenario, numbers of haplotypes present in the native range were higher than in introduced ranges for *A. longifolia*, *A. saligna* and *P. lophantha* (Table 4). However, for *A. cyclops* we identified two

Table 4 Number of haplotypes (N_h) for each study species in both the native and invasive ranges; parentheses show number of haplotypes unique to a specific range

Species	Range	N_h	H	
			$\bar{x}_{pop}$	Total
A. longifolia	Native	3 (2)	0.08	0.75
	Introduced	1 (0)	0.00	0.00
A. melanoxylon	Native	3 (0)	0.40	0.75
	Introduced	4 (1)	0.30	1.29
A. cyclops	Native	2 (1)	0.10	0.48
	Introduced	2 (1)	0.00	0.38
A. saligna	Native	2 (1)	0.23	0.47
	Introduced	1 (0)	0.00	0.00
P. lophantha	Native	5 (3)	0.00	1.37
	Introduced	3 (1)	0.08	0.55

H Shannon's index of diversity (H) is also shown as a population mean ($\bar{x}_{pop}$) and for each range as a whole (total)

haplotypes in both ranges, and lower haplotype diversity in the native versus introduced range of *A. melanoxylon*. Both mean population and total range diversity generally mirrored number of haplotypes, with higher values present in ranges showing the greater number of haplotypes, with the exception of *P. lophantha* where native mean population diversity was zero (i.e. total fixation) despite a high number of haplotypes. In *A. cyclops*, where haplotype diversity was equal in both ranges, the native range showed slightly higher population-level diversity. The introduced ranges of *A. longifolia* and *A. saligna* were comprised of a single haplotype and hence showed no diversity at all (Table 4). Unique haplotypes were found in all native range populations except those of *A. melanoxylon*, and single unique haplotypes were also found in the invasive ranges of *A. melanoxylon*, *A. cyclops* and *P. lophantha*. All invasive ranges shared at least one haplotype with multiple native populations and each invasive haplotype was found in more than one native population, with the exception of the dominant invasive haplotype in *P. lophantha*, which is only present in a single sampled native population (Fig. 1; Table 4).

Discussion

In this study we combined growth-related trait data from a common garden experiment with estimates of genetic diversity derived from populations within the native and introduced range of five Fabaceae species. The common garden experiment showed that there were some significant shifts in growth-related traits between populations from the native and introduced ranges, although these varied among species. Plants of *A. longifolia* from the introduced range had greater biomass and larger SLA than plants from native range populations. For *A. cyclops*, plants from the introduced range had greater RGR, while for *A. melanoxylon*, plants from the introduced range had lower root to shoot ratios. All of these trait changes are likely to result in larger, faster-growing and more competitive plants from introduced compared with native populations, and may be consistent with a shift from defence to growth in response to release from enemies in a novel environment (Jakobs et al. 2004; Rogers and Siemann 2005). Surprisingly however, only for *A. longifolia* did trait differences between native and introduced ranges result in greater biomass, although this may be due to the duration of the experiment. Differences in plant size or biomass is one of the most frequently measured traits in common garden studies, with many observing greater biomass in introduced relative to native populations (Crawley 1987; Bossdorf et al. 2005; Blumenthal and Hufbauer 2007, but see Willis et al. 2000; van Kleunen and Schmid 2003). Our study suggests that only shifts to greater SLA result in greater overall biomass in the introduced range. Interestingly, height and canopy size data from the adult individual plants in this study (Harris and Leishman, unpub. data) confirm that of the five species, only *A. longifolia* plants grow larger in the introduced compared with native range.

A number of previous common garden studies have found differences in traits between native and invasive populations of introduced plants (Bastlová and Kv′t 2002; Bossdorf et al. 2005; Blumenthal and Hufbauer 2007; Zou et al. 2008; Chun et al. 2010; Hodgins and Rieseberg 2011), while other studies have not (e.g. Vila et al. 2003; DeWalt et al. 2004; Guswell et al. 2006). Clearly, there is variability in biomass and leaf trait responses of plants introduced to novel ranges, and our results for five closely-related woody species found that for three species, trait shifts in the introduced range were consistent with shifts to a faster growth strategy, while for two other species this was not the case. By examining chloroplast microsatellite data for these species to determine genetic diversity and infer introduction history, we were then able to assess whether constraints in genetic diversity

are associated with constraints in the adaptive capacity of these species in novel environments.

We found that for three of the five species, genetic diversity was dramatically reduced in the introduced ranges of these species. In agreement with our initial hypothesis, genetic diversity in the introduced range was lower than in the native range for *A. longifolia, A. saligna* and *P. lophantha*, not different for *A. cyclops*, but greater in the introduced range for *A. melanoxylon*. While the links between genetic structure and introduction histories are not always straightforward, especially when dealing with agro-forestry species such as acacias (Le Roux et al. 2011), reductions in genetic diversity for *A. longifolia, A. saligna* and *P. lophantha* suggest two possible scenarios: (1) that genetic bottlenecks and strong drift may have occurred following introductions from restricted parts of the native ranges, or (2) that post-introduction selection eroded genetic diversity. In contrast, the results for *A. melanoxylon* and *A. cyclops* illustrate that no genetic bottleneck was experienced following their introduction, suggesting that there may have been either multiple introductions in the novel range or a single introduction from a diverse source population. Another explanation of the higher diversity in the introduced range of *A. melanoxylon* is that the genetic diversity of this species in its native range was undersampled, possibly due to its very large native range size. However, we suggest that the genetic structure identified within native and introduced ranges for these two species favours multiple introductions as the most likely underlying cause for the patterns observed. Unfortunately, little is known about the movement of most acacias around Australia, presumably because at the time of introduction into their respective introduced ranges, they were considered a native species and thus planting and inter-state importation records were not warranted.

Previous research has found that some Australian acacias exhibit high genetic diversity at the population level in their native ranges (Le Roux et al. 2011), which may lead to locally adapted genotypes. Under this scenario genetic bottlenecks associated with introduced populations may result in a severe reduction in quantitative trait variation and its distribution. Here, the results of the common garden experiment suggest a shift towards faster growth strategies in three of the five species studied, consistent with our initial hypothesis. Our second hypothesis, that genetic variation is lower in the introduced range, was also supported for three of the five species. However, we found no evidence for our third hypothesis that if genetic bottlenecks resulting from introduction constrain invasive potential then species with reduced genetic diversity will not exhibit shifts towards faster growth strategies in the introduced range. Of the three species for which we found evidence of a genetic bottleneck in the introduced range (*A. longifolia, A. saligna* and *P. lophantha*), only *A. saligna* showed no evidence of trait changes while both *A. longifolia* and *P. lophantha* both showed trait changes consistent with a shift towards a faster growth strategy. In contrast, the two species for which standing genetic diversity was comparable or greater in the introduced range (*A. cyclops* and *A. melanoxylon*), did have trait differences that would result in greater aboveground biomass and faster growth in the introduced range. Thus, there does not seem to be a consistent association between changes in genetic diversity and adaptive capacity of these species when introduced to novel environments.

Few common garden studies have also examined genetic diversity of the study species. Dlugosch and Parker (2008) found that increased growth in the introduced range had evolved in the invasive shrub *Hypericum canariense* despite large reductions in genetic diversity. In contrast, Chun et al. (2011) suggested that increased genetic diversity may be associated with rapid adaptation in the invasive annual herb *Ambrosia artemisiifolia.* The lack of a clear association between reduced diversity and reduced adaptive capacity in our study and that of Dlugosch and Parker (2008) may not be unexpected given that adaptive

variation often deviates from expectations based upon neutral genetic variation (Dlugosch and Parker 2008).

Overall, for the five species in this study, shifts in ecological characters between native and introduced ranges appear to be independent of the occurrence of a genetic bottleneck during introduction. Invasion success is likely to be due to multiple causes, not just genetic variation resulting from different introduction pathways. In all studies there is a trade-off between obtaining generality across species and additional detail within species. In this study we were limited to relatively few populations per range per species as we wished to assess the generality of our results across species. Further work is recommended to examine more detailed genetic structure and diversity of exotic species in their native and introduced ranges which would enable assessment of whether populations in the native and introduced range with the same haplotypes have similar phenotypes. Further studies could also assess the level of nuclear genetic variation that has been transferred during introductions.

Acknowledgments We thank Ian Davidson and Anthony Manea for assistance with carrying out the common garden experiment. Thank you also to Christina Birnbaum for assistance in leaf collection for genetic analysis and to all landowners for allowing leaf collection on their land. This research was funded by an ARC Discovery grant DP0879494 to ML.

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