Is rapid evolution common in introduced plant species?

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Summary

1. While previous studies have demonstrated rapid evolution in introduced plants and animals, most focus on single species. They are therefore unable to show whether these are special cases, or if rapid evolution is a common phenomenon in introduced species.

2. We used over 1900 herbarium specimens to determine whether morphological traits [plant height, leaf mass per area (LMA), leaf area or leaf shape] have shown significant change during the last ∼150 years in 23 plant species introduced to New South Wales, Australia.

3. Seventy per cent of our study species showed a change in at least one trait through time. The most common change was in plant height (eight out of 21 species). Six of these showed a decrease in height through time. Decreases in height mainly occurred in western New South Wales where dry, low nutrient conditions may favour shorter plants. We also found changes in leaf traits, including one decrease in LMA, five changes in leaf shape, and three changes in leaf area. The magnitude of these changes was surprisingly large, up to 125% in 100 years.

4. We used specimens of both native Australian species, and of the introduced species taken from their native range to investigate the possibility that the morphological changes were a response to temporal environmental change rather than to the species' introduction to Australia. These control species showed significantly ($P < 0.05$) fewer changes than the introduced species. We included in our analyses information on the region in which each specimen was collected, to account for the possibility that changes through time might be caused by populations radiating in to new environments where a different phenotype might be expressed. Overall, rapid evolution seems the most likely explanation for the changes we observed.

5. *Synthesis.* The majority of our study species showed morphological change through time. While common garden experiments will be required to rule out phenotypic plasticity as an alternative explanation for these patterns, our results suggest that rapid evolution in introduced plant species could be much more common, and of a much greater magnitude than previously thought.

Key-words: Australia, exotic plant, herbarium specimen, invasion ecology, leaf area, leaf mass per area, leaf shape, morphological change, plant height

Introduction

The human migration from Europe to places such as Australia, New Zealand and America that began on a large scale 200 years ago unintentionally set up a huge, replicated transplant experiment. Thousands of plants were introduced to new areas where they became reproductively isolated from their source populations. These plants were freed from many selection pressures faced in their home range, such as coevolved herbivores and parasites. Perhaps most importantly, they were subject to new biotic and abiotic conditions, including a differ-

ent climate, new competitors and new enemies. It is therefore highly likely that these populations of introduced plants are under selection pressure to adapt to the local environmental conditions they face in their new range.

Studies of rapid evolution in plants, birds, insects and mammals show that evolutionary change can occur on timescales of tens to hundreds of years (Cox 2004). Introduced plants have been shown to undergo rapid evolution of dispersal ability (Cody & Overton 1996; Cheptou *et al.* 2008) and reproductive output (Ridley & Ellstrand 2009) as well as phenotypic plasticity (Richards *et al.* 2006), size (Blossey & Notzold 1995; Siemann & Rogers 2001; Bossdorf *et al.* 2004), vegetative reproduction (Lavergne & Molofsky 2007),

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reduced mycorrhizal dependence (Seifert, Bever & Maron 2009) and the formation of genetically based variation along environmental clines in the introduced range (Maron, Elmendorf & Vila 2007).

Most examples of rapid evolution are from studies of single species, and represent a very small proportion of all introduced plant populations. In addition, these examples are likely to be a non-random sample of introduced plant species. Researchers may target study species for which a difference between native and introduced populations has been noticed in the field, or species that are highly invasive. This means that, even collectively, the literature on rapid evolution in introduced species cannot show whether this is the way the majority of plant species respond to changed environmental conditions, or if the examples we have are special cases. Therefore, while we know that rapid evolution is possible and that it is ecologically relevant (Thompson 1998), we still do not know how often it happens. This has important implications for our understanding of how species respond to environmental change. For example, niche conservatism (the tendency for species to retain ancestral characteristics) is often an unstated and untested assumption underlying the bioclimatic models that are widely used to predict species' potential ranges (for example, Peterson 2003; Hartley, Harris & Lester 2006). However, without knowing how common rapid evolution is, we have little understanding of the potential for species to undergo rapid evolutionary change that may allow them to persist or undergo range expansion under novel environmental conditions. Therefore, the main aim of our study was to determine what proportion of plant species undergoes morphological change when introduced to their new range.

The second aim of our study was to find out which morphological traits change, and to determine whether there is a pattern to the direction of this change. Many studies of rapid evolution in introduced plant species have focussed on rapid evolutionary changes caused by biotic processes such as competition and herbivory (Cox 2004; Bossdorf *et al.* 2005). There have been comparatively few studies investigating rapid evolution in response to abiotic environmental conditions, and none in Australian environments. However, there are good reasons to expect that abiotic conditions might impose important selective pressures on introduced plant species.

Many plant functional traits are strongly correlated with environmental conditions. Plants capable of surviving in hotter, drier, nutrient poor conditions tend to be shorter, with smaller, thicker, narrower leaves (Westoby *et al.* 2002; Wright *et al.* 2004; Moles *et al.* 2009). In contrast, plants that perform better in wetter, high nutrient conditions tend to be taller, and have larger, flimsier leaves that maximize their photosynthetic capacity (Westoby *et al.* 2002; Wright *et al.* 2004; Moles *et al.* 2009). Plants growing in many parts of Australia face a hot and dry climate, as well as soils that are often very low in nutrients, particularly phosphorus (Stafford Smith & Morton 1990). Many introduced plants in Australia come from areas such as Europe (Harden 1992) where climate conditions tend to be wetter and cooler, and where soil is generally more fertile.

It is therefore likely that the novel abiotic environmental conditions introduced plants have faced in Australia select for individuals with traits that enhance survival and reproduction under these conditions. The traits we focus on in the present study are plant height and leaf morphology [including leaf size, leaf shape and leaf mass per area (LMA), leaf dry weight divided by leaf area].

Plant height is a central part of plant ecological strategy. It is strongly correlated with life span, seed mass and time to maturity, and is a major determinant of a species' ability to compete for light (Westoby *et al.* 2002; Moles *et al.* 2009). Most studies comparing plant size between introduced and native populations are tests of either the enemy release hypothesis (Keane & Crawley 2002), or the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey & Notzold 1995). The EICA hypothesis proposes that introduced plant populations evolve increased competitive ability (including increased size) when released from specialist herbivores that lived in their home range. Around half of these studies show evidence of increased size in introduced populations, while half show no change (Bossdorf *et al.* 2005). If release from natural enemies drives evolutionary change in introduced species in Australia, we would expect to see increases in plant height. However, if differences in abiotic conditions are the main driver, we would expect to see decreases in plant height, as the species adapt to a drier, more infertile environment.

Leaf mass per area, leaf size and leaf shape, are important determinants of a species' resource acquisition strategy. These traits are correlated with leaf lifespan, photosynthetic rate and susceptibility to herbivory (Westoby *et al.* 2002; Wright *et al.* 2004). Despite their ecological importance, few studies have investigated rapid evolution of leaf traits in introduced plants. Previous studies have found genetically based latitudinal and altitudinal clines in leaf traits in introduced plant populations (Maron, Elmendorf & Vila 2007; Etterson *et al.* 2008) while studies of differences in LMA between introduced and native populations of a species have had mixed results (Güsewell, Jakobs & Weber 2006; Zou, Rogers & Siemann 2007). If biotic factors such as enemy release are driving changes in leaf traits, we predict increases in leaf area and width, and decreases in LMA. However, if the relatively dry and nutrient poor Australian abiotic environment were driving changes in leaf traits, we predict decreases in leaf area and width, and increases in LMA.

We used herbarium specimens to quantify change through time in plant height, leaf area, leaf shape (the ratio of leaf width to leaf length) and LMA in 23 plant species introduced to New South Wales ~150 years ago. Several studies have demonstrated the ability of herbarium specimens to show patterns of morphological change through time (Woodward 1987; McGraw 2001; Law & Salick 2005; Zangerl & Berenbaum 2005), and their use in the study of plant invasion (Crawford & Hoagland 2009). However, herbarium specimens remain an under-utilized resource in ecology. This is the first multi species study of rapid evolution in introduced plants using herbarium specimens.

In summary, we address the following questions:

1 What proportion of plant species introduced to New South Wales shows a significant change in leaf size, shape and/or height since introduction to Australia?

2 Which traits change, and is there a pattern in the direction of these changes?

Answering these questions will increase our understanding of the way rapid evolution shapes species traits in novel environments. This is essential to understanding the role that evolution plays in the invasion process and how species' morphology and distribution is affected by environmental change.

Materials and methods

STUDY SPECIES

We chose species that were introduced to New South Wales before 1920 and have an annual or short-lived perennial life history. This ensures that each species has gone through enough generations in the introduced range for evolutionary change to have occurred. As far as possible, we chose species that are not cultivated and were introduced to New South Wales accidentally. This minimizes the chance that our study populations have been subject to artificial selection. We believe the impact of collection bias on our results is minimal for several reasons. Firstly, by targeting study species whose average size is well within the size of a herbarium sheet, we minimize the risk that smaller individuals in a population will be used as specimens because they fit on a herbarium sheet. Secondly, there is no reason why collection bias would change through time, so collection bias should be random with respect to year of collection.

We measured leaf traits and plant height on herbarium specimens of a total of 23 introduced species. Specimens were collected in New South Wales between 1850 and 2008, and are held at the National Herbarium of New South Wales at the Royal Botanic Gardens, Sydney (NSW) and the Australian National Herbarium at CSIRO in Canberra (CANB). We also measured the same traits on herbarium specimens for species in two control groups: a) five species native to New South Wales (Native Control), and b) three of our introduced species measured in their native range (Home Range Control). Home Range Control specimens were collected in Britain, and are held at Kew Gardens (K), the University of Reading (RNG), and the National Museum of Wales (NMW). We took measurements of Home Range Control species from digital photographs of specimens using Image J (Rasband 1997–2008).

SAMPLING SPECIMENS

For each species, we measured all specimens of the species that each herbarium held from which reliable measurements could be taken. Specimens were not measured if plant traits could not be accurately measured (for example, if too many leaves were damaged, or if plant height could not be measured because only part of the plant was included on the sheet), and where there was no collection date or location. In cases where there was more than one plant on a sheet (i) if we could be sure that the plants were separate individuals, we counted them as separate observations, (ii) if we could not be sure they were separate individuals, we treated them as one individual (this was the case if the plants did not have roots, or if it was characteristic of the species to produce multiple genetically identical individuals connected by a rhizome). We sampled 1–10 leaves on each individual,

depending on the number of leaves the plant produces and the number of undamaged leaves on the specimen. For species with compound leaves, we sampled one leaflet (the terminal leaflet in all cases except in *Vicia villosa*, where we measured the middle leaflet).

REGION OF COLLECTION

As introduced species spread through their new range, they encounter a range of environments. These differences in environmental conditions are likely to affect plant morphology. If plants in our study were collected from different regions of New South Wales in different years, we could potentially mistake morphological differences that are plastic responses to environmental variation for evolutionary change through time. To guard against this possibility, each specimen collected in New South Wales was classified as to whether it was collected in the 'Coast, 'Semi Arid' or 'Arid' region of New South Wales (Fig. 1). These divisions reflect an aridity gradient from east to west in New South Wales as well as botanical divisions within the state. To create these divisions, we amalgamated the following New South Wales botanical divisions (Harden 1992): 'Coast': North Coast, Central Coast, South Coast; 'Semi Arid': North Western Slopes, Central Western Slopes, South Western Slopes, Northern Tablelands, Central Table Lands; Southern Tablelands, 'Arid': North Western Plains, South Western Plains, North Far Western Plains, South Far Western Plains. We accounted for these regions in our statistical analyses for each species (details of analyses below). We did not divide Britain into regions as these were collected in England and Wales, which does not encompass such a wide climatic gradient as New South Wales does.

TRAITS MEASURED

Plant height

We measured plant height on 21 species, either as (i) the distance between the top of the main photosynthetic tissue of the plant and where the roots began, excluding any stems extending above the main foliage, or (ii) the distance between where the roots began and the very top of the plant where the top of the photosynthetic tissue was difficult to distinguish. We took measurements using a tape measure. For prostrate species it is impossible to measure vertical height from a herbarium specimen, and our 'height' measurement is instead a
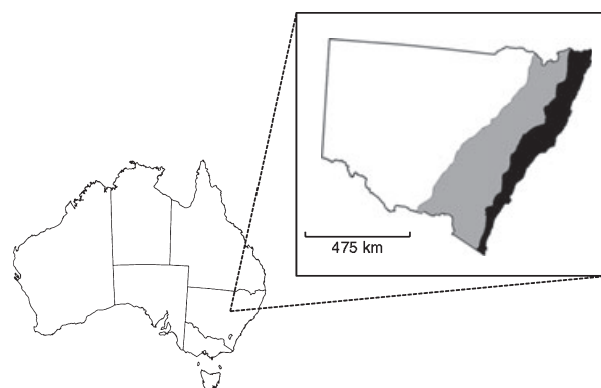
Fig. 1. Map showing the location of the state of New South Wales in Australia, and the delimitation of the three regions used in our study. We divided the state into three regions, 'Coast' (black), 'Semi Arid' (grey), and 'Arid' (white). We included the region in which each herbarium specimen was collected as a factor in our analyses.

measure of the length of the longest stem. However, measurements were always consistent within a species.

Leaf traits: leaf area, leaf shape and leaf mass per area

We measured leaf length and width on 17 species using digital calipers. We measured leaf length as the longest distance in a straight line between the tip and the base of the leaf, excluding the petiole. We measured leaf width as the maximum diameter of the largest imaginary circle that could be fitted on the leaf (Cornelissen *et al.* 2003).

We measured leaf area as the one sided surface area of a leaf excluding the petiole (Cornelissen *et al.* 2003). To calculate leaf area, we used *Image J* (Rasband 1997–2008) to measure length, width and area for one leaf on a digital photo of 7–10 randomly selected specimens per species. The relationship between (leaf length × leaf width) and leaf area was linear with an R^2 value > 0.95 in all cases. We used the slope and intercept of these relationships to estimate leaf area from length and width measurements for each leaf. We calculated leaf shape as the ratio of leaf width to leaf length.

We measured LMA on four species. LMA is calculated as the dry weight of a leaf (including the petiole) divided by its area. For LMA measurements, we measured leaf area using *Image J*. We measured dry weight by weighing each leaf to the nearest hundredth of a milligram. We were limited to measuring only one leaf per individual plant in order to minimize destructive sampling of historical specimens.

STATISTICAL ANALYSIS

Change in trait value through time

For each species and each trait, we used analysis of covariance (ANCOVA) to test the null hypothesis that there was no change in the trait value through time, with year of collection as a covariate and region of collection as a fixed factor. First we fit a model including an interaction term between 'year' and 'region'; if there was no significant interaction ($P > 0.05$) we removed the interaction term and re-ran the analysis. If there was a significant interaction we fitted a linear model for each region separately, using 'year' as the predictor variable. We used linear regression for Home Range Control species, for which we did not include information on region of collection. We would have liked to explore the shape of the relationship between plant morphology and time. This would have allowed us to look at lag phases, and to ask whether evolutionary change was still occurring in the present day, or whether the introduced species had reached new equilibria. However, we did not have sufficient data points to convincingly investigate the shape of these relationships. Therefore, we have performed all analyses with the simplest model: a linear relationship.

These analyses were conducted using JMP version 5 (SAS Institute, Cary, NC, USA). Data were $\log_{10}$-transformed, partly to satisfy assumptions of normality, and partly because the proportional scale recognizes that the same absolute change has different biological significance to plants with high versus low values for that trait (for example, a 1 cm change in height is trivial for a tall tree, but highly important for a short herb).

We applied restrictions to our analyses to guard against spurious results caused by small sample sizes, temporal outliers, or by performing regressions on data points that were only separated by a few years. Species were only included if we had data from at least 25 intact plants, and we performed regressions within a region only where there were 10 or more data points in that region. We did not perform ANCOVA when data points spanned less than 30 years. Similarly, we did

not perform a regression within a region if the data points for that region spanned less than 30 years. We excluded single data points that were isolated temporally from the next closest data point by more than 30 years (we applied this criterion both across regions when analysing regions together, and within regions when performing regressions on data for one region separately).

Weighting

Individuals were the replicates in each analysis. Often multiple individuals are collected at one site and put on a single herbarium sheet. These individuals are likely to be more similar than individuals collected at different sites, both because they grow in the same environment and because they are likely to be more closely related than individuals selected randomly from the population. Therefore, in all analyses we weighted individuals according to the number measured on the herbarium sheet. In cases where there was only one individual per sheet individuals were given a weight of one, whereas in cases where there were two individuals per sheet each individual was given a weight of 0.5. If there were three individuals on one sheet these were given a weight of 1/3, and so on. We chose to weight individuals from the same sheet rather than averaging across the individuals on the sheet because the weighting approach retains all the information about the variance in the data, while correctly allocating degrees of freedom and acknowledging the fact that individuals from the same sheet are not fully independent.
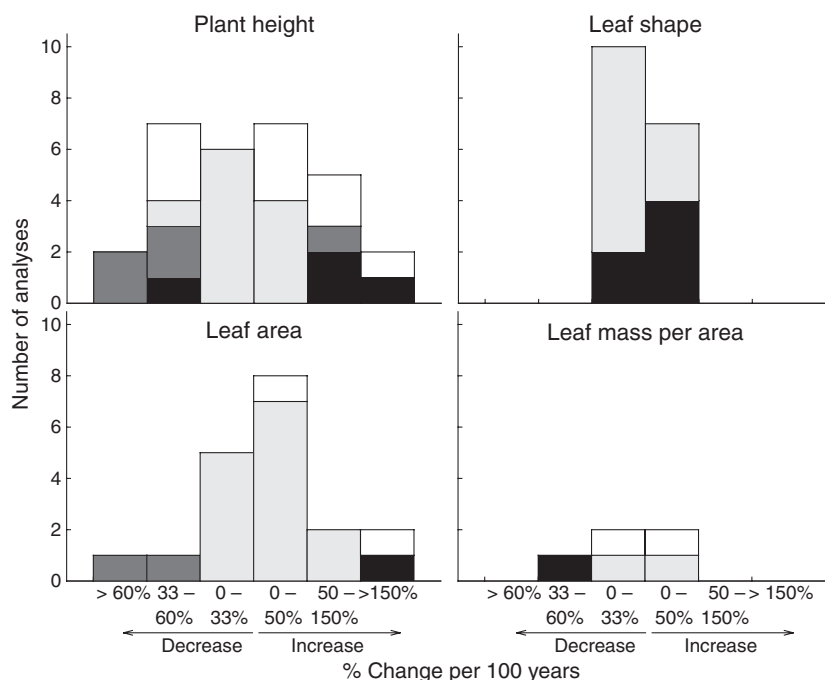
Results

Sixteen of the 23 introduced species measured (70%) showed a change in at least one trait through time. The largest change was a decrease in plant height of 125% per 100 years in *Facelis retusa*. Table S1 in Supporting Information gives results for all species. Histograms showing the magnitude of all changes are shown in Fig. 2.

Eight out of 21 introduced species showed a change in plant height (Fig. 3). Six of these species showed a decrease in height through time. *Aira cupaniana*, *Aphanes arvenesis*, *Trifolium glomeratum*, *Silene gallica* and *Polycarpon tetraphyllum* showed a decrease in height in the Semi Arid region, while *Facelis retusa* showed a decrease in height across all regions. *Arctotheca populifolia* showed an increase in height in the coast region, and *Veronica arvensis* showed an increase in height across all regions. One Native Control species, *Trigonella suavissima*, showed a change in plant height through time (Fig. S1 in Supporting Information). The largest change in plant height was a decrease of 125% per 100 years in *Facelis retusa*, closely followed by a decrease of 121% in *Aphanes arvensis*. No Home Range Control species show a significant change in plant height through time (Fig. S2 in Supporting Information).

Three out of 17 species showed a change in leaf area (Fig. 4). Two species showed a decrease in leaf area through time, *Cicendia quadrangularis* in the Semi Arid region and *Medicago lupulina* in the Coast region. *Veronica arvensis* showed an increase in leaf area across all regions. The largest change in leaf area was a decrease of 78% per 100 years in *Cicendia quadrangularis*, followed by a decrease of 35% in *Medicago lupulina*.

Fig. 2. Histogram showing per cent change per 100 years for each species in plant height, leaf shape, leaf area, and leaf mass per area (LMA). Dark shades represent significant results, while light shades represent non-significant results. Significant results in analyses across all plants for a species are shown in black; significant results within region are shown in dark grey; non-significant results across all plants for a species are shown in light grey and non-significant results for analyses within a region are shown in white.

Five out of 17 species showed a change in leaf shape (Fig. 5). Three species showed an increase in the ratio of leaf width to leaf length through time, *Geranium molle* and *Lotus corniculatus* across all regions and *Arenaria leptoclados* in the Semi Arid region. Two species, *Trifolium glomeratum* and *Gamochaeta americana* showed a decrease in leaf width to length ratio across all regions. The largest change in this ratio was an increase of 19% per 100 years in *Geranium molle*, followed by a 17% decrease in *Gamochaeta americana*.

One of four species, *Euphorbia peplus*, showed a change in LMA through time across all regions (39% per 100 years, Fig. 6). One Home Range control species, *Trifolium dubium* showed an increase in the ratio of leaf width to leaf length through time (Fig. S2). No Native Control species showed a significant change in leaf traits through time (Fig. S1).

Because we performed a large number of statistical tests we used a binomial test to determine whether the number of significant results was greater than would be expected by chance with an alpha of 0.05. We performed 124 tests for our introduced species, 26 of which were significant. The probability of observing this many or more significant results due to chance alone is extremely low ($P < 0.001$). In contrast, the number of significant tests for control species was not significantly more than would be expected by chance (2 out of 30 tests, $P = 0.45$). Finally, a chi-squared contingency analysis showed that the number of introduced species showing a change through time was significantly more than the number of control species showing a change ($P = 0.02$, $\chi2 = 5.7054$,

df = 1.1). This analysis rules out the possibility that the observed changes in introduced species in Australia were simply a response to global climatic change, or environmental changes in Australia over the last 150 years, and provides some support for the idea that the changes in introduced species are an evolutionary response to their new environment.

Discussion

While the number of studies demonstrating rapid evolutionary change in introduced species has increased in recent years, we lacked information about how common this phenomenon actually is. Seventy per cent of our 23 study species showed evidence of morphological change over the past 150 years since introduction to a new environment in at least one of the four traits studied. The magnitude of these changes was surprisingly high, the largest being 125% per 100 years. These findings suggest that rapid evolution could be remarkably common in introduced plant species. The capacity for short-term evolution is likely to enhance the spread of introduced plant populations in new areas. For native species, it may facilitate persistence in existing range locations in the face of a changing climate.

PLANT HEIGHT

Ten out of 21 species showed a change in plant height through time. Of these, six are decreases in height. These results follow

Fig. 3. Plant height measured on 21 plant species introduced to New South Wales. Each data point represents one individual. Individuals collected in the 'Coast' region are indicated by black dots, in the 'Semi Arid' region by grey triangles, in the 'Arid' region by open squares. *P*, *R*² and slope values are given where we found significant change through time. Eight species showed a change in plant height. Six species showed a decrease in height through time. *Aira cupaniana*, *Aphanes arvensis*, *Trifolium glomeratum*, *Silene gallica*, and *Polycarpon tetraphyllum* showed a decrease in height in the Semi Arid region, while *Facelis retusa* showed a decrease across all regions. *Arctotheca populifolia* showed an increase in height in the coast region, and *Veronica arvensis* showed an increase in height across all regions.
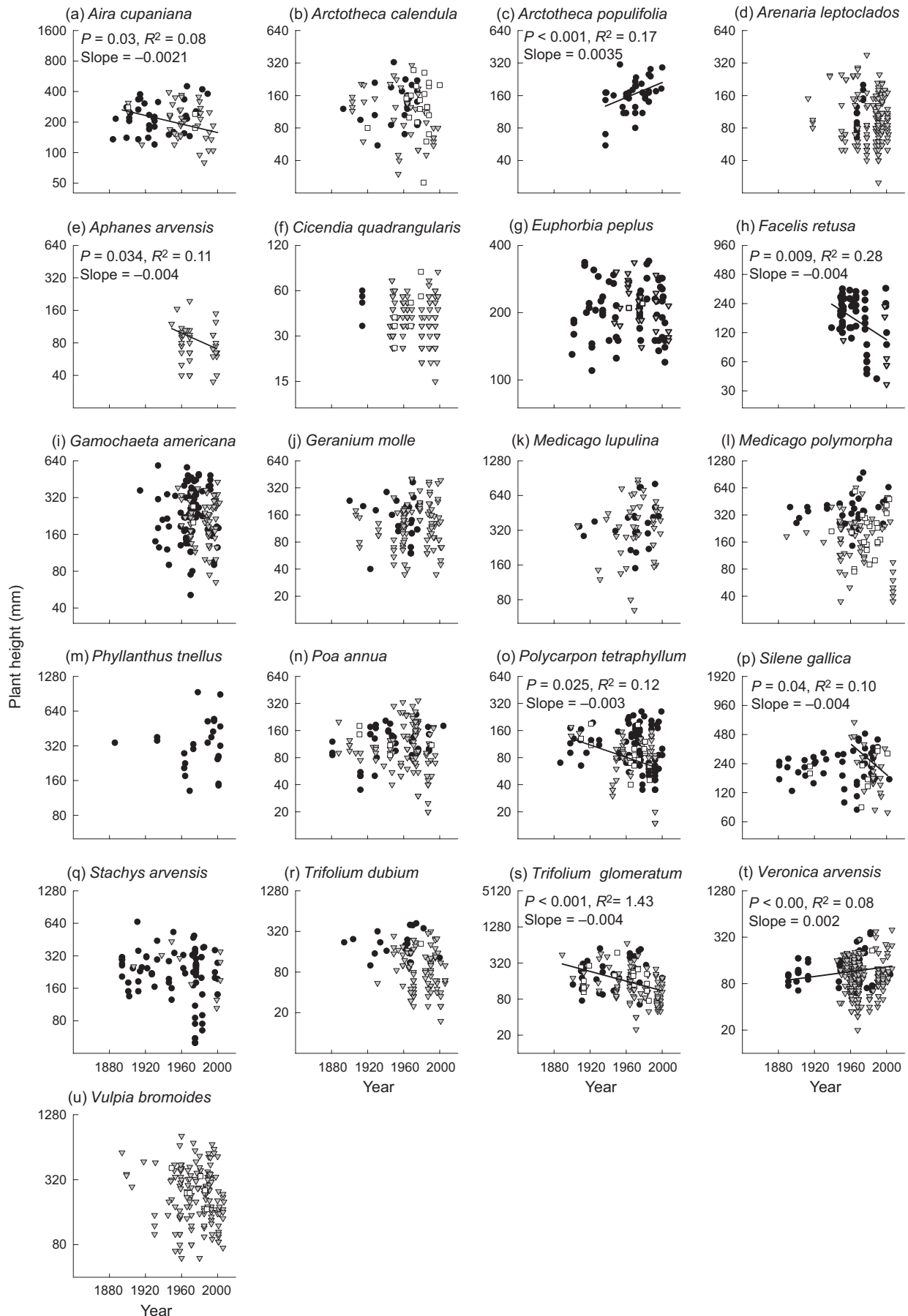
a completely different pattern to that predicted by the EICA hypothesis (Blossey & Notzold 1995), and to that found in the literature, where around half of all studies looking for size differences between native and introduced populations show increased size in the introduced population while the other half find no change (Bossdorf *et al.* 2005). These studies used common environment experiments and usually did not include competitors. It has been shown that the outcome of such studies can be strongly affected by the presence of competitors (Bossdorf *et al.* 2004; Blumenthal & Hufbauer 2007). The fact that the plants in our study were growing in the presence of competitors (being wild plants collected in the field) is one potential explanation for the difference in these patterns.

Another possibility is that abiotic conditions were more important than biotic release for the species in our study. The majority of our observed decreases in height occurred in arid western New South Wales, while height in the higher rainfall coastal New South Wales tended to show either no change, or increase through time. This pattern is consistent with the idea that the more arid environmental conditions in western New South Wales have selected for decreased height in this area, while competition plays a greater role in the more mesic coastal region. Anthropogenic nutrient enrichment and water addition are also more intense in the more densely-populated coastal regions (Leishman, Hughes & Gore 2004; Leishman & Thomson 2005), and these factors might further reduce abiotic selection pressure on introduced populations.

Differences between our results and those in the literature could also be due to a bias in study species chosen for previous studies of rapid evolution. Researchers examining size differences between native and introduced populations may be more likely to choose study species for which there is an observed increase in height in the introduced range. There could also be a 'file drawer' effect, where studies that find an opposite trend to that expected are less likely to be published than those which corroborate a researcher's hypothesis (Rosenthal 1979).

LEAF TRAITS

We found fewer changes in leaf traits than plant height. Only one of four species, *Euphorbia peplus*, showed a change in LMA through time (a decrease). This trend is the opposite of what we expected on the basis of environmental conditions. High LMA is associated with low nutrient soils, low rainfall, and high temperatures (Westoby *et al.* 2002; Wright *et al.* 2004), as found in New South Wales. However, *E. peplus* grows mostly in highly nutrient enriched habitats in New South Wales, such as gardens and footpaths (Harden 1992). In these habitats selection pressures imposed by the abiotic environment are relaxed. The decrease in LMA could be explained by the EICA Hypothesis: release from coevolved herbivores and parasites might have lead to selection for traits that favour increased growth rate. Importantly, the observed pattern clearly rules out deterioration of herbarium specimen leaves over time as an explanation. Previous studies comparing LMA between native and introduced populations have mixed results.

Güsewell, Jakobs & Weber (2006) found no significant difference in LMA between native and invasive populations of *Solidago gigantea*, while Zou *et al.* (2006) found lower LMA in invasive compared to native populations of *Sapium sebiferum*.

Five out of 17 species showed a change in leaf shape through time, and three out of 17 showed a change in leaf area. Unlike plant height, there is no pattern in the direction of these changes. This is the first study of leaf shape in introduced plant species. Previous studies have found latitudinal and altitudinal clinal variation in leaf area in introduced plant species, which can be genetically based (Parker, Rodriguez & Loik 2003; Maron, Elmendorf & Vila 2007; Etterson *et al.* 2008), indicating adaptation to local climate conditions. Our results suggest that changes in leaf traits in introduced plant species occur less frequently than changes in plant height.

Overall, our findings suggest that the direction of evolutionary change in introduced plants is context dependent. Rather than always being increases in size as the result of biotic release, or always being driven by adaptation to environmental conditions, the direction of change most likely depends on whichever of these is more limiting to plant growth.

COULD THE OBSERVED CHANGES BE DUE TO PHENOTYPIC PLASTICITY?

Plants grown in different environments (as in the home versus new ranges of an introduced plant species) may differ morphologically because of phenotypic responses to the different conditions, rather than as the result of evolutionary change in response to their new environment. However, in this study, we track change through time in the same range (either the introduced or the home range for each species), rather than by comparing morphology from plants in contrasting environments. Therefore, the changes we observed are not a simple phenotypic response to the difference between growing conditions in Australia and the growing conditions in the species' home ranges.

A second possibility is that a change in morphology through time could be observed as a population spreads along an environmental gradient, if the plants showed a phenotypic response to the different environmental conditions. For instance, a trend towards decreasing height through time might be observed as species moved westward in New South Wales over time and collections were made from increasingly arid areas. To guard against this possibility, we recorded the geographic region in which each sample plant was collected, and included a term for region in our analyses. We only pooled data from plants from different regions where analysis showed that region did not have a significant effect. That is, the significant results we report have accounted for differences in region. It is still possible that the introduced species occupied different habitats through time within the major geographic regions. However, the lack of detail in collection location records for many of the older herbarium specimens prevents us from investigating this idea.

Finally, any environmental changes (such as global climate change or local changes in land use in Australia) over the past
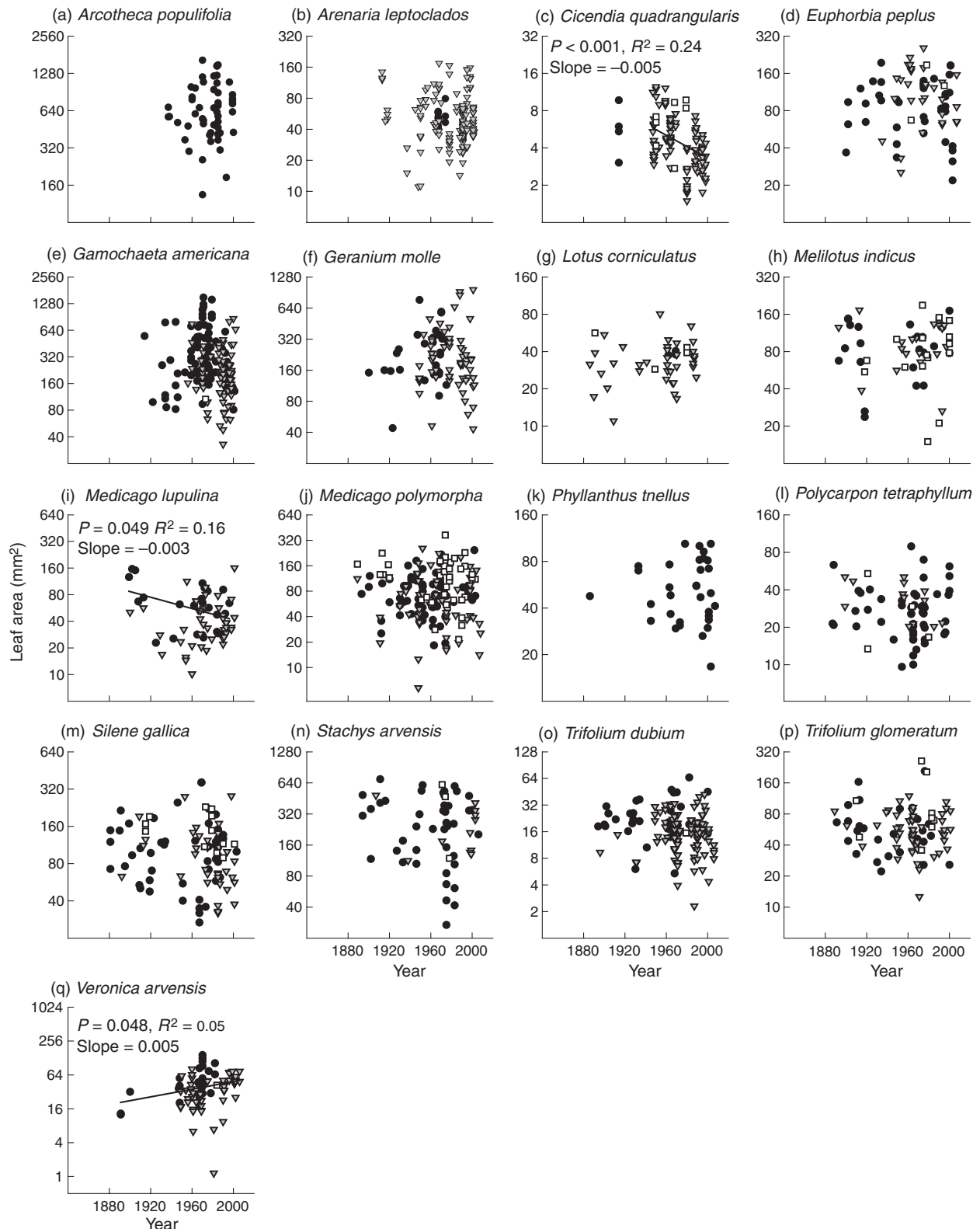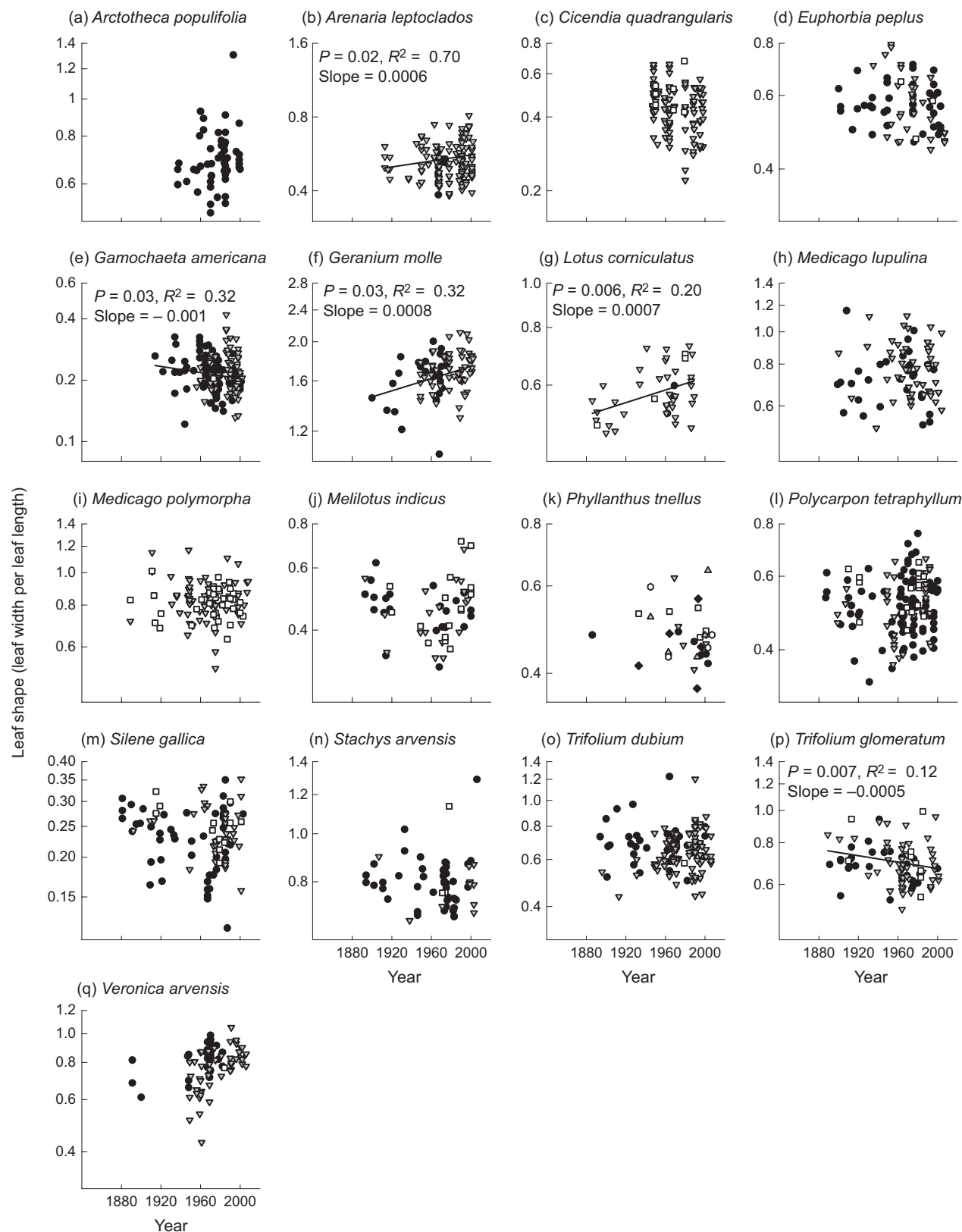
Fig. 4. Leaf area measured on 17 plant species introduced to New South Wales. P, R^2 and slope values are given where we found significant change through time. Three species showed a change in leaf area. Two species showed a decrease in leaf area through time, *Cicendia quadrangularis* in the Semi Arid region and *Medicago lupulina* in the Coast region. *Veronica arvensis* showed an increase across all regions. Symbols as in Fig. 3.

150 years could influence plant morphology. To determine whether environmental change might be responsible for the observed changes in our introduced species, we investigated

change in eight control species (five native Australian plants and three of the species that had been introduced to Australia, sampled from their home range). The fact that we found

Fig. 5. Leaf shape (the ratio of leaf width to leaf length) measured on 17 plant species introduced to New South Wales. P, R^2 and slope values are given where we found significant change through time. Five species showed a change in leaf shape. *Geranium molle,* and *Lotus corniculatus* showed an increase in leaf width to length ratio across all regions. *Arenaria leptoclados* showed an increase in this ratio in the Semi Arid region). *Gamochaeta americana* and *Trifolium glomeratum* showed a decrease in leaf width to length ratio across all regions. Symbols as in Fig. 3.
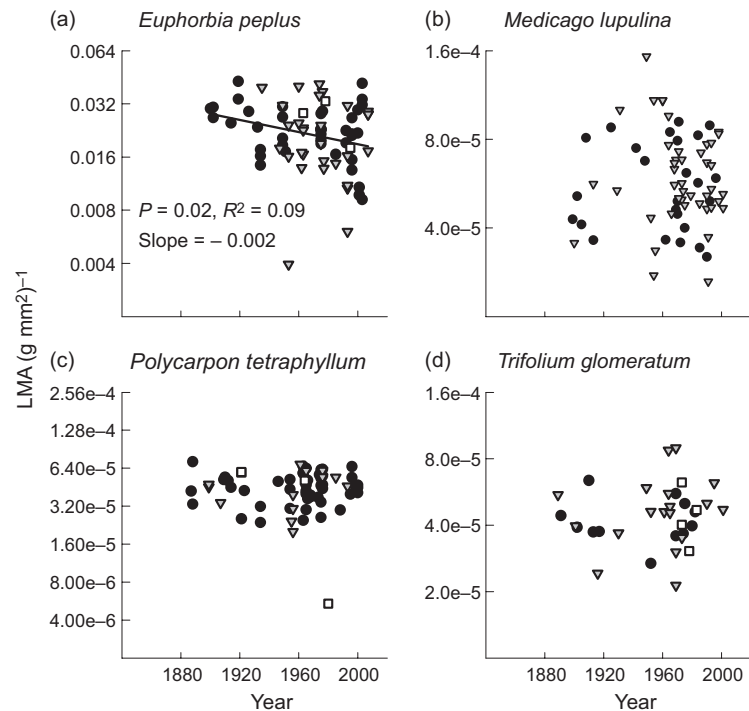
Fig. 6. Leaf mass per area (LMA) measured on four plant species introduced to New South Wales. P, R^2 and slope values are given where we found significant change through time. We found a significant change in LMA through time in one species, *Euphorbia peplus*, which showed a decrease in LMA across all regions. Symbols as in Fig. 3.

significantly fewer changes in our control species than in our introduced species suggests that the changes we observed are not due to environmental change through time. The fact that we did observe a few changes in native plant species in New South Wales is consistent with the fact that these areas have experienced many environmental changes as the result of anthropogenic disturbance over the last 150 years (including exposure to introduced species). There are also many examples of native species evolving in response to introduced species (Strauss, Lau & Carroll 2006).

In summary, we did as much as possible to rule out the possibility that the changes we observed are due to phenotypic plasticity. It seems likely that these changes are indeed a result of rapid evolution in response to the novel conditions the introduced species encountered in Australia. However, we cannot definitively prove that the changes are not due to phenotypic plasticity without performing common garden experiments using seed from both native and introduced populations of these species. We plan to begin such experiments in the near future.

Conclusion

Our study suggests that rapid evolutionary change in response to novel environmental conditions is likely to be much more common than previously thought. The magnitude of the changes we observed, up to 125% in 100 years, is surprisingly large, certainly large enough to have ecological implications. The direction of these changes (especially those in plant height) is consistent with the idea that abiotic environmental conditions impose selection pressure on these populations. As introduced species become better adapted to their new environments, they are likely to become stronger competitors and more successful invaders. Thus, rapid evolution in response to local environmental conditions could play a role in facilitating invasions by introduced plants. On a more positive note, our results suggest that many plant populations may have the potential to adapt *in situ* to environmental change. However, our study species all have short generation times so represent plants with the highest potential for short term evolutionary change: rates for longer-lived species could be much lower. The next step will be to ask what factors we can use to predict the likelihood that a species will evolve rapidly in response to environmental change. This will enhance our ability to predict which plant species are likely to become invasive if introduced to new areas, or which species might still be here a few hundred years from now.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Results of tests for trait change through time in introduced plant species in Australia, (a) Introduced species, (b) Native Control species, (c) Home Range Control species.

Figure S1. Plant height, leaf shape and leaf area measured on Native Control species.

Figure S2. Plant height, leaf shape and leaf area measured on Home Range Control species.

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