



NOTICE OF MEETING

**Notice is hereby given of the Meeting of the
Bluff Community Board
to be held in the Bluff Municipal Chambers,
Gore Street, Bluff on
Monday 9 March 2020 at 7.00 pm**

Mr R Fife (Chairperson)
Mrs G Henderson (Deputy Chair)
Mr G A Laidlaw
Mr N Peterson
Miss T Topi
Cr A J Arnold

CLARE HADLEY
CHIEF EXECUTIVE

AGENDA

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2. APOLOGIES	
3. PUBLIC FORUM	
3.1 SOUTH PORT UPDATE	
Mr Nigel Gear, Chief Executive, Mr Frank O'Boyle, Infrastructure Manager and Mr Jamie May, Business Development Manager, will be in attendance to speak to this item.	
4. INTEREST REGISTER A2411889	4
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9. BLUFF COMMUNITY BOARD CHAIRMAN'S REPORT To be tabled.	
10. URGENT BUSINESS	
10.1 CONCERNS ABOUT TREE REMOVAL	19

11. **PUBLIC EXCLUDED SESSION**

Moved, seconded that the public be excluded from the following parts of the proceedings of this meeting; namely

- (a) *Confirmation of Minutes of Public Excluded Session Minutes of Meeting – 3 February 2020.*

The general subject of each matter to be considered while the public is excluded, the reason for passing this resolution in relation to each matter, and the specific grounds under Section 48(1) of the Local Government Official Information and Meetings Act 1987 for the passing of this resolution are as follows:

General subject of each matter to be considered	Reason for passing this resolution in relation to each matter	Ground(s) under Section 48(1) for the passing of this resolution
(a) Confirmation of Minutes 3 February 2020	Enable any local authority holding the information to carry on, without prejudice or disadvantage, negotiations (including commercial and industrial negotiations)	Section 7(2)(i)

BLUFF COMMUNITY BOARD INTEREST REGISTER

ELECTED MEMBERS		
NAME	ENTITY	INTERESTS
Cr Allan Arnold	Invercargill City Council	Councillor
	Ziff's Café Bar Ltd	Executive Director
	Buster Crabb Ltd	Executive Director
	Ziff's Tour Ltd	Executive Director
	Ziff's HR Ltd	Executive Director
	Ziff's Trust	Trustee Administrator
	NZMCA	Member
	Southland Aero Club	Member
	Invercargill Club	Member
Invercargill East Rotary	Member	
Cr Peter Kett	Invercargill City Council	Councillor
Raymond Fife	Bluff Community Board	Board Member
	Bluff Charitable Trust	Chairman
	Bluff Maritime Museum	Chairman

Bluff Community Board - INTEREST REGISTER

Gloria Henderson	Bluff Community Board Bluff Maritime Museum Bluff Promotions Charitable Trust Bluff Women's Section of RSA Hospice	Deputy Chairperson Deputy Chairperson Vice Chairperson Trustee RSA Member
Graham Laidlaw	Bluff Community Board	Board Member
Noel Peterson	Bluff Community Board	Board Member
Tammi Topi	Bluff Community Board	Board Member

MINUTES OF A MEETING OF THE BLUFF COMMUNITY BOARD HELD IN THE BLUFF MUNICIPAL CHAMBERS, GORE STREET, BLUFF ON MONDAY 3 FEBRUARY 2020 AT 7.00 PM

PRESENT: Mr R Fife (Chair)
Mrs G Henderson (Deputy Chair)
Mr G Laidlaw
Mr N Peterson
Miss T Topi
Cr A J Arnold

IN ATTENDANCE: His Worship the Mayor
Mr R Pearson – Roading Manager
Mrs D Fife – Assistant Manager - Bluff Service Centre
Ms M Frey – Interim Parks and Reserves Manager
Mr L Beer – Bluff Publicity/Promotions Officer
Ms L Kuresa – Committee Secretary

2. **APOLOGIES**

Nil.

3. **PUBLIC FORUM**

3.1 **10 Year Plan and the New Trust Set-up**

Cherie Chapman was in attendance to speak to this item.

Ms Chapman spoke about how the 10 Year Plan could be improved. She took the Board through a visual presentation with ideas on how to progress the Plan. She suggested having someone from outside Bluff, who would be keen to be a member of the new Bluff Enterprise Arts Trust to be part of a collaborative approach that could be led by the Murihiku Arts Trust.

The Board was impressed with the ideas presented by Ms Chapman and agreed that there would be a need to access funding to develop the ideas.

In response to a question, Ms Chapman explained that it was a five year plan and some taskforce groups could be approached to carry out the work. She said that there was a gentleman from the Arts Trust who could be approached to lead this project as it was good to have an outside person to lead it.

The Chairman thanked Ms Chapman for taking the time to present to the Board.

4. **INTEREST REGISTER**

Moved N Peterson, seconded G Henderson and **RESOLVED** that the Interest Register be received.

5. **MINUTES OF MEETING HELD 18 NOVEMBER 2019**

Moved G Henderson, seconded T Topi and **RESOLVED** that the minutes be approved with the correction on Page 7 of the agenda that it should be N Peterson and not N Patterson.

6. **REPORT OF THE BLUFF PUBLICITY/PROMOTIONS OFFICER**

Moved Cr Arnold, seconded G Laidlaw that the report be received.

The report had been circulated and Mr Beer took the meeting through it.

It was confirmed that the Southland Annual Cheeseroll event that was to be held 16 February had been postponed due to the low number of entries.

The motion, now being put, was **RESOLVED** in the **affirmative**.

7. **ACTION SHEET**

Moved T Topi, seconded G Laidlaw that the report be received.

Mr Pearson and Ms Frey took the meeting through the report.

Mr Pearson reported on the following items not on the report:

1. An intrusive investigation work of the Ocean Beach site would be carried out on 17 February, which would involve diggers at a number of places to identify the extent of the landfilling of that site. A beach pick up was carried out pre-Christmas with another one planned to determine the materials that had been deposited across that site.
2. Not much work was being done to the Cycle Track as there was an Archaeological Authority process being undertaken and they were 22% through the assessment.

Ms Frey reported that Parks Week was planned from 7 March to 14 March. She noted that the Summer Sounds would be held 8 March and she would contact Mr Beer and propose that it be promoted through Invercargill City Council website. There were a series of initiatives for Parks Week with a big focus to tie it in with local events happening in the community. She informed the Board that the water fountain had arrived which would be installed soon.

The Chairman reported that the Board had received some emails with regard to cutting down some of the big pine trees on Bluff Hill. N Peterson commented that it was wrong to cut down 150 year old tree and he would submit to the next Board meeting evidence to show that. It was agreed that he would liaise with Ms Frey and provide more information about it.

In response to a question, Mr Pearson explained that they would follow up with the electrical contractor as to why the lights on the Christmas tree were not working on Christmas day.

The Chairman said that a number of people had commented that there was a lack of Christmas decorations put up last year with some looking "tatty". Mr Pearson explained that some of the decorations did not sustain the wind very well. He would be putting a report to the Executive Team outlining the budget for Christmas trees and decorations. There was a need to review what needed to be done and when it would be done to ensure that the budget was consistent and appropriate.

The Chairman raised the issue of sump cleaning and street sweeping and what progress had been made. Mr Pearson explained he had received some information on other areas that he was not satisfied with the contractor performance as they needed to keep going back to do the work. There was a need to demonstrate that they were here every two months carrying out the work.

G Henderson raised the issue of the spraying along the main street, which had not been done for a long time. Mr Pearson explained that spraying in Bluff would be happening soon. He had asked that GPS be installed in the trucks, so that he was aware when the work was being carried out.

G Henderson noted that there was no signage at the dog park and there was no parking area. She also wondered if the owners were using the park because rocks were an issue when accessing the dog park. She suggested that this item be include in the Tour of Inspection as it was not user-friendly.

The motion, now being put, was **RESOLVED** in the **affirmative**.

8. **BLUFF COMMUNITY BOARD CHAIRMAN'S REPORT**

The report was tabled and the Chairman took the meeting through it.

8.1 **Community Board Bursaries**

The Chairman reported that the Committee interviewed four applicants for the Community Board Bursaries and awarded:

- Rachel Ryan who was studying Early Childhood at the Otago College of Education in Invercargill.
- Shayla Habberfield who would be studying Pre-professional Social Work at the University of Otago.

A good range of applications were received and the Board wished everyone best wishes for future studies.

8.2 **Tour of Inspection**

The Chairman said that a date in March would be confirmed for the Tour of Inspection.

8.3 **Community Board Induction Workshop**

The Chairman reported that this workshop was carried out with Mike Reid from Local Government New Zealand. It was good way to keep up with what was happening around the country.

8.4 **Meeting with Local Police**

The Chairman said that the Board met with the local Police before Christmas regarding the lawlessness around Bluff.

N Peterson informed the Board that there was a community support programme available that he would like to bring a report to the next Board Meeting, which the Board agreed to.

Moved R Fife, seconded G Henderson and **RESOLVED** that the report be received.

9. **FINANCIAL STATEMENT**

The report had been circulated.

Moved G Laidlaw, seconded N Peterson and **RESOLVED** that the report be received.

9. **URGENT BUSINESS**

9.1 **Coronavirus**

His Worship the Mayor asked if there had been an update to the Coronavirus.

The Chairman said that there had been some concern around Bluff being an international port with regard to ships that travel the world with multiple stops in China. There were also ships that stopped off in Bluff that were manned by Chinese crew. Concerns had been raised with Nigel Gear, the Chief Executive Officer of South Port and they were awaiting the outcome of that.

After discussions, it was agreed that the situation was being viewed closely and the Chairman would keep in touch with the appropriate people to stay up to date with what was happening.

10. **PUBLIC EXCLUDED SESSION**

Moved N Peterson, seconded G Henderson and **RESOLVED** that the public be excluded from the following parts of the proceedings of this meeting, namely:

- (a) *Confirmation of Minutes held 18 November 2019.*
- (b) *Action Sheet – In Committee Items.*

The general subject of each matter to be considered while the public is excluded, the reason for passing this resolution in relation to each matter, and the specific grounds under Section 48(1) of the Local Government Official Information and Meetings Act 1987 for the passing of this resolution are as follows:

General subject of each matter to be considered	Reason for passing this resolution in relation to each matter	Ground(s) under Section 48(1) for the passing of this resolution
(a) Action Sheet – In Committee Items	Enable any local authority holding the information to carry on, without prejudice or disadvantage, negotiations (including commercial and industrial negotiations)	Section 7(2)(i)
(b) Confirmation of Minutes – 18 November 2019	Enable any local authority holding the information to carry on, without prejudice or disadvantage, negotiations (including commercial and industrial negotiations)	Section 7(2)(i)

TO: BLUFF COMMUNITY BOARD

FROM: LINDSAY BEER - BLUFF PUBLICITY/PROMOTIONS OFFICER

MEETING DATE: MONDAY 9 MARCH 2020

REPORT OF THE BLUFF PUBLICITY/PROMOTIONS OFFICER

RECOMMENDATIONS

It is recommended that this report be received.

STAR INSURANCE BURT MUNRO CHALLENGE BLUFF HILLCLIMB – 6 FEBRUARY 2020

This year's hillclimb went well although ticket sales on the day were down, one factor no doubt being the flooding that occurred in the lead up to the event. Being the first event we probably took the brunt of that crowd wise, a shame as being on a public holiday for the first time we were looking forward to our best crowd ever.

Some of our volunteers were also caught up in the floods and had to cancel although our volunteer numbers this year meant that there were no issues around this.

As previously reported the expectation this year was that we took on more work around the event to get gear to Bluff and set up the course ready for motorcycle club representatives to come and inspect it as opposed to in the past just assisting the club with this. We were able to do this as well as carrying out the dismantling of the course at the end of the day of the event and return all the gear to Invercargill in time for the street race as we have always done.

While volunteers on the day were not such an issue due to the public holiday it was still difficult to find assistance on the day before the event. We took advantage of the South Port NZ Ltd community engagement programme with two of their staff helping in Invercargill to load gear on Wednesday morning and then one staff member with a Manitou at the Bluff end. I was also loaned a ute and a salvage trailer (courtesy of Challenge Newfield) which proved invaluable.

The only problems we encountered were some transport issues to and from Invercargill and a report that someone delivering meds could not access a property within our road closure area. I have discussed that with the person concerned. There was also a diesel spill in several places on the road on race morning believed to have come from a campervan. However the Bluff Fire Brigade quickly dealt with the situation.

I am indebted to many people and groups for their assistance as the event is a large logistical exercise. Bluff Engineering & Welding and Osborn Engineering in particular are a great help in dismantling the course and assisting with the loading of gear to get it back to Invercargill while the extra assistance from South Port was invaluable also. There are several individuals and groups that assist as well and we will make donations to several groups in the near future.

A thank you in the Beacon has been printed and we will repeat this on Facebook.

SUMMER SOUNDS BLUFF – SUNDAY 8 MARCH 2020

Summer Sounds will have been completed by the time of this meeting. We engaged three acts this year – Darcy Kerr and Emmy Roderique from Bluff and Radiowave from Invercargill. Advertising was placed on social media along with the Southland Express, Bluff Beacon and Whats On Invers. I am indebted to Creative Communities, Bluff Engineering & Welding, i Hire Invercargill and DOC for their assistance with the event.

ST JOHN BLUFF BOOK SALE / FLOWER SHOW

I carried out some publicity for both these events prior to each.

TRY-WHANAU TRIATHLON – SUNDAY 22 MARCH 2020

I have put out an initial media release on this event which has thus far featured in Whats On Invers and Southland Express.

TO: BLUFF COMMUNITY BOARD
FROM: RUSSELL PEARSON, ROADING MANAGER AND MICHELE FREY, ACTING PARKS MANAGER
MEETING DATE: MONDAY 9 MARCH 2020

BLUFF ACTION SHEET

SUMMARY

This report provides an update on the Action Sheet.

RECOMMENDATION

That the report “Bluff Action Sheet” be received.

IMPLICATIONS

1.	<i>Has this been provided for in the Long Term Plan/Annual Plan?</i> Yes.
2.	<i>Is a budget amendment required?</i> No.
3.	<i>Is this matter significant in terms of Council’s Policy on Significance?</i> No.
4.	<i>Implications in terms of other Council Strategic Documents or Council Policy?</i> None.
5.	<i>Have the views of affected or interested persons been obtained and is any further public consultation required?</i> Not required.
6.	<i>Has the Child, Youth and Family Friendly Policy been considered?</i> N/A.

FINANCIAL IMPLICATIONS

No financial issues have been raised.

ACTION SHEET

A number of projects are either underway or have been completed in the Bluff community, which is reflected in the attached spreadsheet (refer to **Appendix 1**). In addition, a verbal update will be provided to the Board at this meeting.

Bluff Community Board - ACTION SHEET

APPENDIX 1

A2930071

Bluff Inspection Action Sheet

Item	Year	Responsibility Area	Issues	Actions	ICC Contact	Anticipated Timeframe	Completed
4	2018	Parks	Rowing club beach erosion	No further current action but does highlight risks around filled areas and erosion.	Parks	Hold	
6	2018	Parks	J G Ward Reserve	Vehicles are using this area as access to the back of the sections. One property has put a new gate onto the Reserve for access and Parks have received complaints. There are now 4-5 properties using this illegal access onto a Reserve. This needs the Board's support and residents asked to stop. Community Board are going to have a discussion and advise following discussion.	Parks	Hold awaiting response	
2	2019	Roading	Gore Street Road Verge (fuel tanks)	Parks, Mobil and the 2024 Group are working together to develop a plan for the area. Discussion held on how the area will continue to be maintained after new plantings are installed. Suggestion that a grant could be provided to give time for future costs to be considered in the Annual Plan. Comments about certainty of funding. Further work with the planned cycleway and the work from the 2024 Group needs to be incorporated into a wider plan for the area. Cycle trail design still being completed.	Russell Pearson	Nov-19	
3	2019	Roading	Foreshore Road by Shannon Street	Lupins had been sprayed and area could need a tidy up. Contractor to check. Check being undertaken to make sure spraying has been effective.	Russell Pearson	Complete	
4	2019	Parks	Boat Ramp – Swimming Area	Detailed design of boat ramp underway, sub-division of land progressing, tender documentation being compiled.	Lesley McCoy	TBC - Dependant on tenders	
6	2019	Roading	Stirling Point	Footpath is still awaiting decisions. Parking Options Study is planned by Stafford Group. Vegetation around the viewing platform needs a tidy. Light at sign is damaged and needs repaired. Contractor instructed to repair light. Report to be discussed at future workshop with Board and Councillors.	Russell Pearson	Hold	
7	2019	Roading	Gun Pit Road Carpark	DOC keen to work with Council on a carparking option and DOC planning further improvements. Further investigation needed into what carparking is possible. Road into the area is unsealed and needs to be sealed if used as carpark. Concepts are contained in the Strategic Plan. This work needs to be tied into Stirling Point actions. Meeting held with DOC but needs some planning to tie into a funded plan.	Russell Pearson	Hold	
8	2019	Roading	Gorse in Roadsides	Areas visited which have gorse starting to grow, including Bann and Barrow Street. Roding to follow up. More spraying and remedial work is required and follow up needed. Contractor will be instructed to recheck berm areas and respray as necessary. Next round of spraying is being undertaken and further audits will occur. The kerb side spray is finished and the noxious spray is due in November.	Russell Pearson		
9	2019	Parks	Bluff War Memorial	Subsidence of ground under the memorial, a report has been undertaken for options. Next steps are to consult with community about options.	Michele Frey		
10	2019	Parks	Information Kiosk	Steering group established with members from Bluff Community Board, Bluff 2024, Bluff promotions and looking to find member from Bluff Motepohue Trust. Detailed design is underway. Steering groups working with Parks staff on funding application document. Progressing well.	Lesley McCoy		
11	2020	Parks	Stirling Point Lighthouse Track	Repairs are underway.	Lesley McCoy	Feb-20	
12	2020	Parks	Gore Street Playground	Largely complete. Seats to be installed in coming weeks. Trialling new type of seat.	Lesley McCoy	Feb-20	
13	2020	Parks	Skate Park Water Fountain	Installation about to commence. Dependent on weather conditions for concrete pouring.	Michele Frey		

Bluff Refuse Transfer Site

Malcolm Loan explained the compactor would be transferred from Invercargill
Green waste is processed when there is sufficient material, which could be a
Gore Street Road Verge (Fuel tanks)

Mobil and the 2024 Group are working together to develop a plan for the area
Discussion held on how the area will continue to be maintained after new plan
Suggestion that a grant could be provided to give time for future costs to get
Comments about certainty of funding

- Foreshore Road by Shannon St
Lupins had been sprayed and area could need a tidy up
- Boat Ramp – Swimming Area
Current boat ramp funding does not include any swimming options
Discussion on the current parking of boats adjacent to the ramp which will not
Discussed the ES Clean Hull Boat Maintenance area and that ES was funding it
It was noted that the Swimming Concept Area is included in the Bluff Strategic Plan
- Gore Street – Hedge by Morrison’s Beach
Agreed that a major trim would be undertaken before a decision is made to trim
- Stirling Point
Footpath is still awaiting decisions
Parking Options Study is planned by Stafford Group
Vegetation around the viewing platform needs a tidy up
Light at sign is damaged and needs repaired
- Gun Pit road Carpark
DOC keen to work with Council on a carparking option and DOC planning further
Further investigation needed into what carparking is possible
Road into the area is unsealed and needs to be sealed if used as carpark
Concepts are contained in the Strategic Plan
- Gorse in Roadsides
Areas visited which has gorse starting to grow, including Bann and Barrow St
Roading to follow up
- Sweeping Frequency
Some areas look to have not been adequately swept
Some grass still in the channels

gill. It will have more capacity than the current. The compactor is owned by the Contractor.
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TO: BLUFF COMMUNITY BOARD

FROM: SANIYA THOMPSON, COMMUNITY DEVELOPMENT OFFICER

MEETING DATE: MONDAY 9 MARCH 2020

NEIGHBOURHOOD SUPPORT PROGRAMME
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SUMMARY

The Community Development Officer has been asked by a Bluff Community Board Member to present to the Board on the development of the Neighbourhood Support Programme in Bluff.
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RECOMMENDATIONS

That the report “Neighbourhood Support Programme” be received.

IMPLICATIONS

1.	<i>Has this been provided for in the Long Term Plan/Annual Plan?</i> N/A
2.	<i>Is a budget amendment required?</i> N/A
3.	<i>Is this matter significant in terms of Council's Policy on Significance?</i> N/A
4.	<i>Implications in terms of other Council Strategic Documents or Council Policy?</i> N/A
5.	<i>Have the views of affected or interested persons been obtained and is any further public consultation required?</i> N/A
6.	<i>Has the Child, Youth and Family Friendly Policy be considered?</i> Yes

FINANCIAL IMPLICATIONS

N/A.

THE NEIGHBOURHOOD SUPPORT PROGRAMME

Neighbourhood Support, formerly known as Neighbourhood Watch, is an international initiative that appeared as a response to crime in the late 60s and gained popularity in the UK, USA, Australia and New Zealand. Today in New Zealand, Neighbourhood Support is a nationwide community development initiative that brings neighbours together to create safer and more connected neighbourhoods.

In 2004, the Invercargill City Council and Police joined forces to revitalise Neighbourhood Support locally. The project was seen as an effective way to reduce crime and to promote safer communities in the city.

Setting up a Neighbourhood Support group is a great way to improve safety, resilience and well-being of residents. Members of groups are encouraged to share and regularly update their contact information, have meetings and social get-togethers, share information to reduce crime and fear of crime, and look out for one another; especially those who are more vulnerable. Groups can vary in size and the activity of each group is unique – there is no “one size fits all”.

There are presently over 100 Neighbourhood Support Groups in Invercargill.

Advantages of belonging to a group include:

- Getting to know your neighbours and being able to contact them via a group phone or email list
- Street signs may deter burglars and other offenders
- Information and alerts on local crime and scams
- Information on improving household and neighbourhood safety
- Peace of mind knowing that your neighbours are keeping an eye on your property when you are away (e.g. at work or on holiday)
- Support for more vulnerable neighbours (e.g. elderly or disabled)
- Information on how to be better prepared for an emergency situation

There are three Neighbourhood Support Groups operating in Bluff; one is still active as a group and the other two consist of individual members. At the moment we are not actively seeking areas to open new groups, but respond when there is an interest in the community.

Is rapid evolution common in introduced plant species?

Joanna M. Buswell^{1,2*}, Angela T. Moles¹ and Stephen Hartley²

¹Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, The University of New South Wales, NSW 2052, Australia; and ²School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand

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 trans-plant 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 time-scales 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; '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

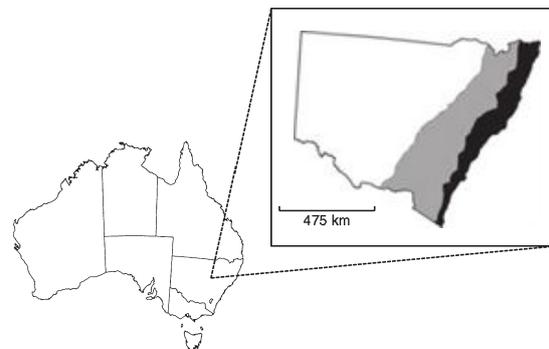


Fig. 1. Map showing the location of the state of New South Wales in Australia, and the delimitation of the three regions used in our study. We divided the state into three regions, 'Coast' (black), 'Semi Arid' (grey), and 'Arid' (white). We included the region in which each herbarium specimen was collected as a factor in our analyses.

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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 \times 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 reran 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 arvensis*, *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*.

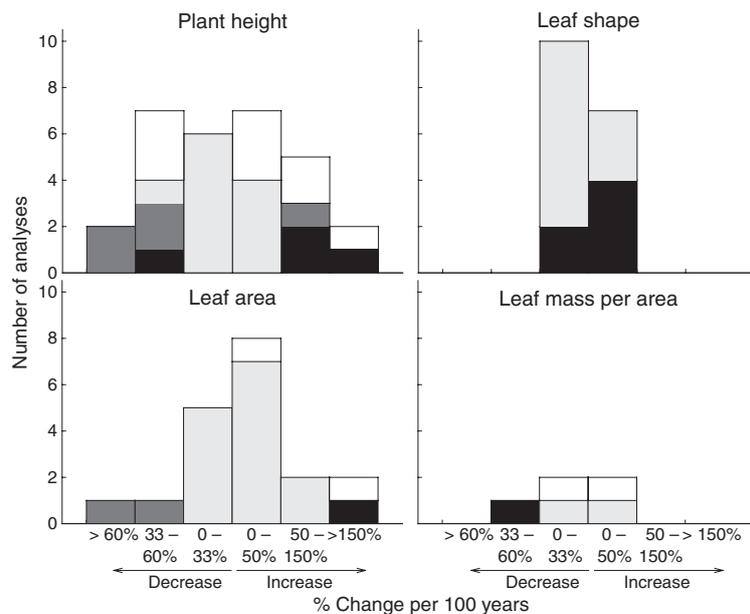


Fig. 2. Histogram showing per cent change per 100 years for each species in plant height, leaf shape, leaf area, and leaf mass per area (LMA). Dark shades represent significant results, while light shades represent non-significant results. Significant results in analyses across all plants for a species 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 pepus*, 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 ($P = 0.02$, $\chi^2 = 5.7054$, $df = 1.1$). 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.

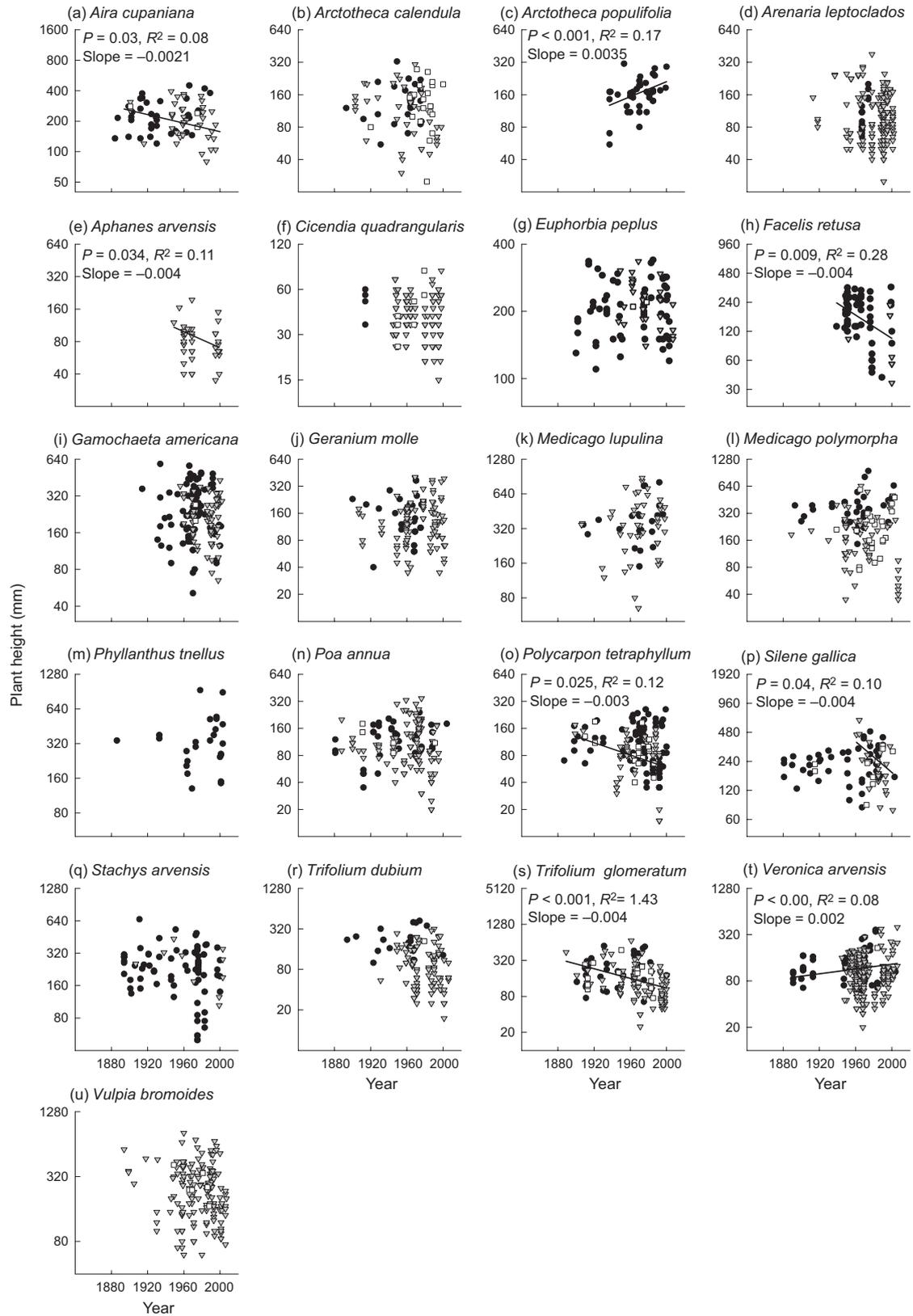
Discussion

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 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.

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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 led 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 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. 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.

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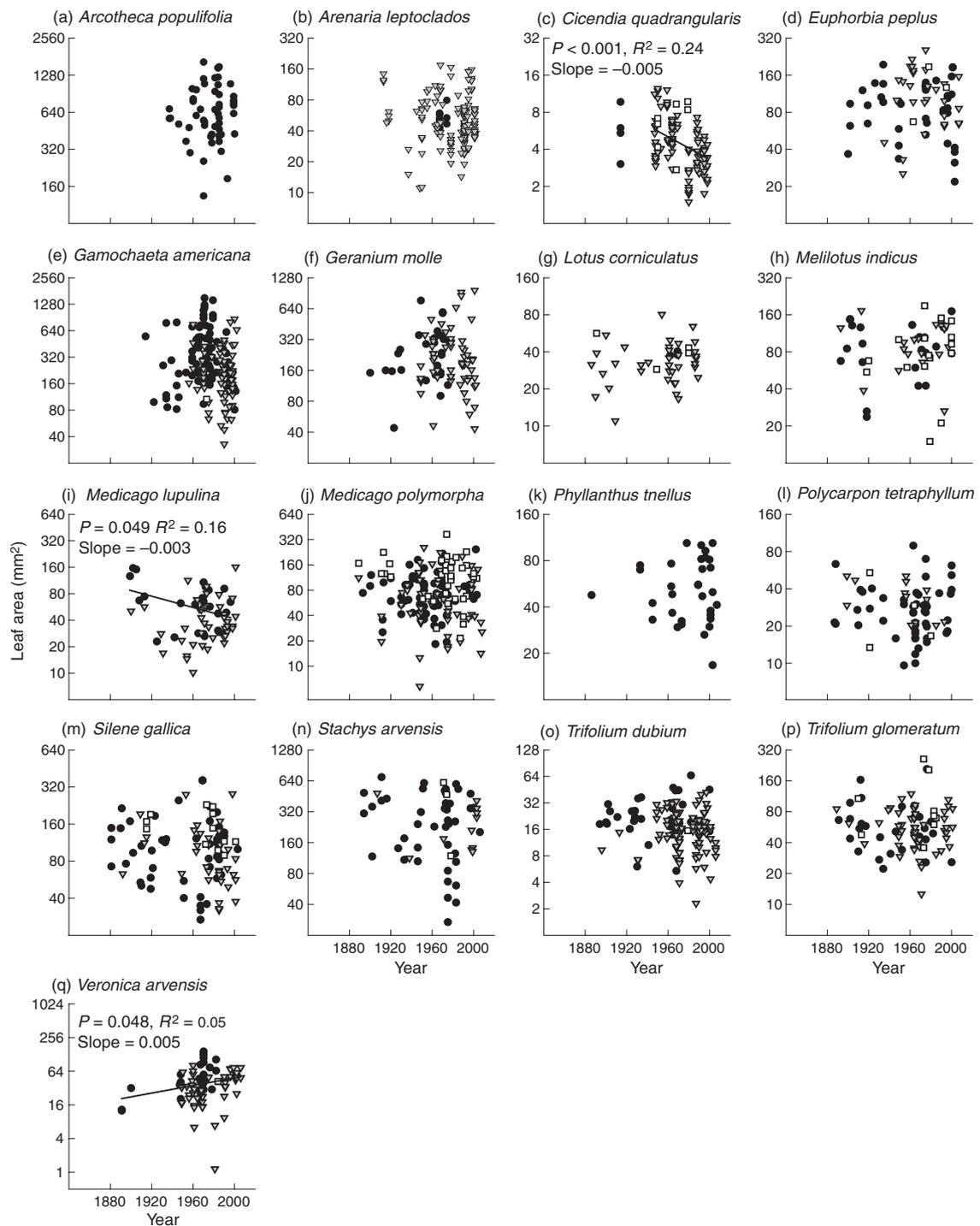


Fig. 4. Leaf area measured on 17 plant species introduced to New South Wales. P , R^2 and slope values are given where we found significant change through time. Three species showed a change in leaf area. Two species showed a decrease in leaf area through time, *Cicendia quadrangularis* in the Semi Arid 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

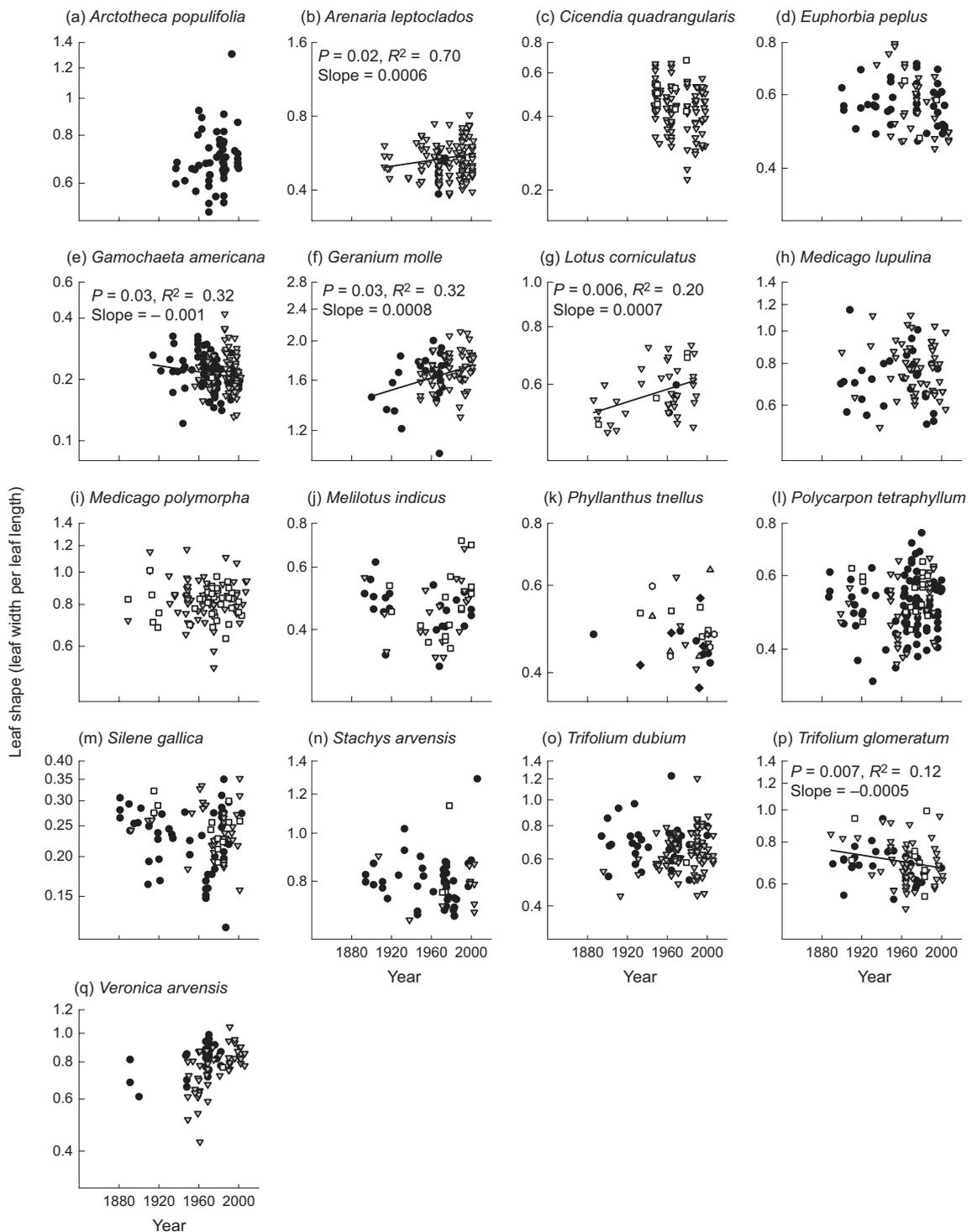


Fig. 5. Leaf shape (the ratio of leaf width to leaf length) measured on 17 plant species introduced to New South Wales. P , R^2 and slope values are given where we found significant change through time. Five species showed a change in leaf shape. *Geranium molle*, and *Lotus corniculatus* showed an increase 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.

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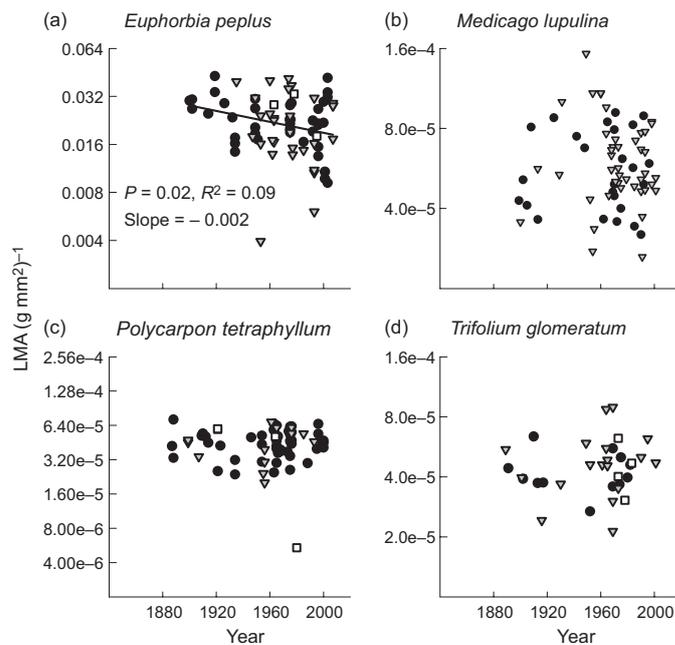


Fig. 6. Leaf mass per area (LMA) measured on four plant species introduced to New South Wales. P , R^2 and slope values are given where we found significant change through time. We found a significant change in LMA through time in one species, *Euphorbia peplus*, which showed a decrease in LMA across 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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References

- Blossey, B. & Notzold, R. (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology*, **83**, 887–889.
- Blumenthal, D.M. & Hufbauer, R.A. (2007) Increased plant size in exotic populations: a common garden test with 14 invasive species. *Ecology*, **88**, 2758–2765.
- Bossdorf, O., Prati, D., Auge, H. & Schmid, B. (2004) Reduced competitive ability in an invasive plant. *Ecology Letters*, **7**, 345–353.

- 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.
- Cheptou, P.O., Carrue, O., Rouifed, S. & Cantarel, A. (2008) Rapid evolution of seed dispersal in an urban environment in the weed *Crepis sancta*. *Proceedings of the National Academy of Sciences*, **105**, 3769–3799.
- Cody, M.L. & Overton, J. (1996) Short-term evolution of reduced dispersal potential in island plant populations. *Journal of Ecology*, **84**, 53–61.
- Cornelissen, J.H.C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G. & Poorter, H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335–380.
- Cox, G.W. (2004) *Alien Species and Evolution*. Island Press, Washington.
- Crawford, P.H.C. & Hoagland, B.W. (2009) Can herbarium records be used to map alien species invasion and native species expansion over the past 100 years? *Journal of Biogeography*, **36**, 651–661.
- Etterson, J.R., Delf, D.E., Craig, T.P., Ando, Y. & Ohgushi, T. (2008) Parallel patterns of clinal variation in *Solidago altissima* in its native range in central USA and its invasive range in Japan. *Botany*, **86**, 91–97.
- Güsewell, S., Jakobs, G. & Weber, E. (2006) Native and introduced populations of *Solidago gigantea* differ in shoot production but not in leaf traits or litter decomposition. *Functional Ecology*, **20**, 575–584.
- Harden, G.J. (1992) *Flora of New South Wales*. New South Wales University Press, Sydney.
- Hartley, S., Harris, R. & Lester, P.J. (2006) Quantifying uncertainty in the potential distribution of an invasive species: climate and the Argentine ant. *Ecology Letters*, **9**, 1068–1079.
- Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, **17**, 164–170.
- Lavergne, S. & Molofsky, J. (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 3883–3888.
- Law, W. & Salick, J. (2005) Human-induced dwarfing of Himalayan snow lotus, *Saussurea laniceps* (Asteraceae). *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 10218–10220.
- Leishman, M.R., Hughes, M.T. & Gore, D.B. (2004) Soil phosphorous enhancement below stormwater outlets in urban bushland: spatial and temporal changes and the relationship with invasive plants. *Australian Journal of Soil Research*, **42**, 197–202.
- Leishman, M.R. & Thomson, V.P. (2005) Experimental evidence for the effects of additional water, nutrients and physical disturbance on invasive plants in low fertility Hawkesbury Sandstone soils, Sydney, Australia. *Journal of Ecology*, **93**, 38–49.
- Maron, J.L., Elmendorf, S.C. & Vila, M. (2007) Contrasting plant physiological adaptation to climate in the native and introduced range of *Hypericum perforatum*. *Evolution*, **61**, 1912–1924.
- McGraw, J.B. (2001) Evidence for decline in stature of American ginseng plants from herbarium specimens. *Biological Conservation*, **98**, 25–32.
- Moles, A.T., Warton, D.I., Warman, L., Swenson, N.G., Laffan, S.W., Zanne, A.E., Pitman, A., Hemmings, F.A. & Leishman, M.R. (2009) Global patterns in plant height. *Journal of Ecology*, **97**, 923–932.
- Parker, I.M., Rodriguez, J. & Loik, M.E. (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Conservation Biology*, **17**, 59–72.
- Peterson, A.T. (2003) Predicting the geography of species' invasions via ecological niche modelling. *Quarterly Review of Biology*, **78**, 419–433.
- Rasband, W.S. (1997–2008) *Image J*. National Institute of Health, <http://rsb.info.nih.gov/ij/>, Bethesda, Maryland.
- Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters*, **9**, 981–993.
- Ridley, C.E. & Ellstrand, N.C. (2009) Evolution of enhanced reproduction in the hybrid-derived invasive, California wild radish (*Raphanus sativus*). *Biological Invasions*, **11**, 2251–2264.
- Rosenthal, R. (1979) The “file drawer problem” and tolerance for null results. *Psychological Bulletin*, **86**, 638–641.
- Seifert, E.K., Bever, J.D. & Maron, J.L. (2009) Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. *Ecology*, **90**, 1055–1062.
- Siemann, E. & Rogers, W.E. (2001) Genetic differences in growth of an invasive tree species. *Ecology Letters*, **4**, 514–518.
- Stafford Smith, D.M. & Morton, S.R. (1990) A framework for the ecology of arid Australia. *Journal of Arid Environments*, **18**, 225–278.
- Strauss, S.Y., Lau, J.A. & Carroll, S.P. (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, **2006**, 357–374.
- Thompson, J.N. (1998) Rapid evolution as an ecological process. *Trends in Ecology & Evolution*, **13**, 329–332.
- Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology, Evolution and Systematics*, **33**, 125–159.
- Woodward, F.I. (1987) Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature*, **327**, 617–618.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F. et al. (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821–827.
- Zangerl, A.R. & Berenbaum, M.R. (2005) Increase in toxicity of an invasive weed after reassociation with its coevolved herbivore. *Proceedings of the National Academy of Sciences*, **102**, 15529–15532.
- Zou, J., Rogers, W.E. & Siemann, E. (2007) Differences in morphological and physiological traits between native and invasive populations of *Sapium sebiferum*. *Functional Ecology*, **21**, 721–730.
- Zou, J.W., Rogers, W.E., DeWalt, S.J. & Siemann, E. (2006) The effect of Chinese tallow tree (*Sapium sebiferum*) ecotype on soil-plant system carbon and nitrogen processes. *Oecologia*, **150**, 272–281.

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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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Rapid reshaping: the evolution of morphological changes in an introduced beach daisy

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Thousands of species have been introduced to new ranges worldwide. These introductions provide opportunities for researchers to study evolutionary changes in form and function in response to new environmental conditions. However, almost all previous studies of morphological change in introduced species have compared introduced populations to populations from across the species' native range, so variation within native ranges probably confounds estimates of evolutionary change. In this study, we used micro-satellites to locate the source population for the beach daisy *Arctotheca populifolia* that had been introduced to eastern Australia. We then compared four introduced populations from Australia with their original South African source population in a common-environment experiment. Despite being separated for less than 100 years, source and introduced populations of *A. populifolia* display substantial heritable morphological differences. Contrary to the evolution of increased competitive ability hypothesis, introduced plants were shorter than source plants, and introduced and source plants did not differ in total biomass. Contrary to predictions based on higher rainfall in the introduced range, introduced plants had smaller, thicker leaves than source plants. Finally, while source plants develop lobed adult leaves, introduced plants retain their spatulate juvenile leaf shape into adulthood. These changes indicate that rapid evolution in introduced species happens, but not always in the direction predicted by theory.

1. Introduction

Over 13 000 vascular plant species have become naturalized in new ranges worldwide [1]. These introductions have resulted in many ecological impacts on resident species and ecosystem processes [2]. Increasingly, the evolutionary impacts of introductions are also becoming apparent: introduced species can promote evolutionary diversification in native species, and undergo evolutionary changes themselves [3]. These evolutionary changes can occur rapidly (often within tens to hundreds of generations [4]) and have been demonstrated in a wide range of introduced plants and animals in terrestrial, aquatic and marine environments. Some well-known examples include cane toads in Australia [5], zebra mussels in the USA and Europe [6], and smooth cordgrass on the West Coast of the USA [7].

Common-environment experiments are often used to test for evolutionary changes in introduced species [8]. However, in all but one [9] previous study

of morphological change between introduced and native populations of plants, the actual source population for the introduction has been unknown. Without knowing the original source of an introduction, we cannot accurately assess what evolutionary changes have taken place, because the use of native plants from a broad range introduces variation that may obscure differences between native and introduced populations (figure 1). This affects how we interpret differences found between native and introduced plants, and is likely to be especially problematic in cases where introduced plants have large native distributions and thus span wide environmental and/or biotic gradients. To overcome these limitations, we located the source population for *Arctotheca populifolia*, a beach daisy that was introduced to Australia from South Africa in the 1930s [10]. We set up a common-environment experiment to compare plants from the known source population in South Africa with plants from four introduced populations in Australia. We examined the evidence of evolutionary divergence after introduction using a suite of six plant growth traits and six leaf traits, to capture information about resource acquisition strategy and life-history strategy [11,12].

Introduced species can undergo evolutionary changes via three mechanisms: hybridization, natural selection or genetic drift [3]. Hybridization between introduced and native species is an important process that has been demonstrated in several previous studies (e.g. [7]), but is not occurring in *A. populifolia* in Australia (there are no native *Arctotheca* in Australia [13]). Genetic drift can cause evolutionary changes in introduced populations [14], and may be a significant factor for *A. populifolia* because it has been shown to have one of the lowest levels of genetic diversity for an introduced species [15], but it is usually not possible to predict the direction of these changes. However, we can arrive at several hypotheses for the ways in which natural selection might lead to morphological change in *A. populifolia*.

Selection for morphological change could arise from differences in the abiotic environment between the source and introduced ranges. The source and introduced populations of *A. populifolia* are at similar latitudes and experience similar temperatures, but rainfall in the introduced range is two to three times higher than in the source location (electronic supplementary material, appendix S1: table S1). There is generally a positive relationship between rainfall and plant size [16], so we predicted that the higher rainfall in Australia would lead plants from the introduced populations of *A. populifolia* to be larger than plants from the source population. Specifically, we predicted that the Australian plants would have greater total and above-ground biomass, and be taller and longer than their South African counterparts. The one size trait that we did not expect to be greater in Australia was below-ground biomass. We predicted that the introduced plants would allocate a greater percentage of their resources above-ground than below-ground in response to higher water availability [17]. Wetter conditions might also favour the evolution of larger, thinner, higher specific leaf area (SLA) leaves with lower dry matter content and low tissue density in the introduced range [11,12,18]. However, we did not predict a difference in leaf shape. Leaf shape affects leaf thermal properties [19], but there is little difference in temperature between the source and introduced range.

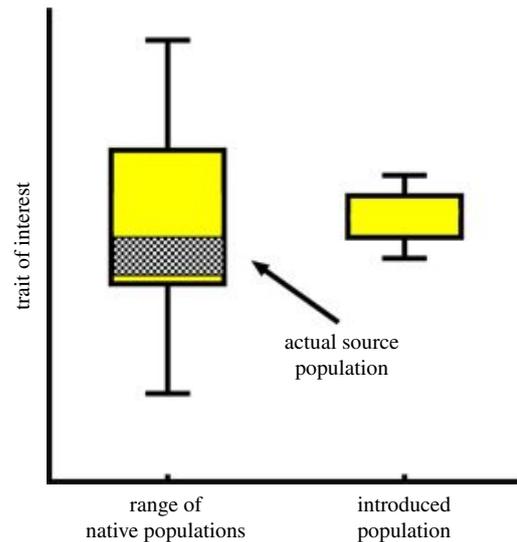


Figure 1. Sampling widely across the range of native populations can add variation that could lead to differences between the actual source population and the introduced population becoming obscured or underestimated. (Online version in colour.)

Selection for morphological change could also arise as a result of differences in biotic interactions between the source and introduced range. The evolution of increased competitive ability (EICA) hypothesis proposes that release from specialist herbivores allows introduced plants to shift resources from defence into growth and reproduction, and so evolve increased competitive ability and larger size [20]. We do not yet have data on the degree of herbivory experienced by *A. populifolia* in its native versus source ranges, but if enemy release is occurring, we would expect the plants to grow larger in Australia (taller and longer, with greater total, above-ground and below-ground biomass). While leaf shape can affect apparency to herbivores [21], we have no specific predictions as to how leaf shape might change in response to decreased herbivory. However, smaller, tougher leaves are less vulnerable to herbivory [18,22], so reduced herbivory in the introduced range might allow the evolution of larger, thinner, higher SLA leaves with lower dry matter content and low tissue density [12,18].

Biotic and abiotic selective pressures are not mutually exclusive. In the case of *A. populifolia* in Australia, selective pressures from enemy release and increased rainfall are predicted to act in the same direction, favouring increases in plant size and larger, higher SLA leaves (predictions summarized in table 1). The only trait considered here for which biotic and abiotic selective pressures are predicted to have opposing effects is below-ground biomass (increasing under EICA, decreasing in response to higher rainfall; table 1). Further, there are other mechanisms through which plant traits might change, including genetic drift and differences in other, unmeasured selective pressures between the native and introduced range. Thus, we cannot definitively prove which mechanisms underpin any observed changes. However, we can determine whether there have been evolutionary changes in plant traits between the source and introduced populations of *A. populifolia*, and observe whether any trait changes are broadly consistent with the collective predictions based on EICA and rainfall.

Table 1. Summary of predictions and outcomes for each trait. Predictions are based on (1) EICA theory and (2) higher rainfall in Australia. ‘+’ indicates a prediction for greater values of the trait in Australia, ‘-’ indicates a prediction for lower values of the trait in Australia and ‘.’ indicates no difference (or no particular prediction). The fourth column gives the observed results for each trait (details in figure 1 and figure 2). Only five of the 24 predictions (indicated by asterisks) were consistent with observed results.

trait	prediction under EICA	prediction based on higher rainfall in Australia	observed
total biomass	+	+	.
plant length	+ *	+ *	+
plant height	+	+	-
plant length/height	.	.	-
above-ground biomass	+	+	.
below-ground biomass	+	- *	-
SLA	+	+	.
leaf dry matter content	-	-	.
leaf area	+	+	-
leaf density	- *	- *	-
leaf thickness	-	-	+
leaf shape	.	.	-

2. Methods

(a) Study species

Arctotheca populifolia (P.J. Bergius) Norlindh is a perennial, semi-succulent herb in the Asteraceae. It is a coastal pioneer species native to South Africa where it is grows on the foredunes of sandy beaches and is common along the coastline [23]. The earliest records of this species in Australia date back to the 1930s, and it is now present in two separate regions: the east coast and southwest Western Australia [10].

Location of the source population for the *Arctotheca populifolia* populations in eastern Australia was based on microsatellite data collected by Rollins *et al.* [15], complemented by new analyses (details in electronic supplementary material, appendix S2). Briefly, these data give four indications that the east Australian populations of *A. populifolia* are most closely aligned with a population from Arniston in southwest South Africa: STRUCTURE analysis; principal component analysis; differentiation measured by R_{ST} (electronic supplementary material, appendix S2); and the fact that Arniston is the only South African population that includes all of the rare alleles found in the east Australian plants. These indications that Arniston is the source demand formal statistical comparison of two hypotheses: that Arniston is, or is not, the source of the east Australian plants. Therefore, we performed log odds analyses (LOD, electronic supplementary material, appendix S2) which indicate that Arniston is at least 10^{99} times more likely to be the source population than any other South African population (despite our making the tests conservative, i.e. biased against making such a conclusion; see electronic supplementary material, appendix S2). Thus, it seems reasonable to conclude that Arniston is the source for the east Australian invasion.

(b) Collection locations

We collected seeds from four locations spanning approximately 600 km on the east coast of Australia (Mallacoota, Narooma, Wairo Beach and Treachery Beach; electronic supplementary material, appendix S1) in February 2011, and from the source population in Arniston, South Africa in April 2011. These locations correspond to those used in the microsatellite study

[15]. At each location, seeds from multiple seed heads on individual plants were collected. The number of individuals sampled at each location ranged from 17 to 46 (electronic supplementary material, appendix S3, table S3a).

(c) Minimizing possible maternal effects

To avoid confounding maternal effects with genetic effects when comparing our populations, we used the seeds collected in the field to grow parent plants which then produced a standardized generation of offspring for our main experiment. The maternal environment in which seeds are produced can affect several plant traits in the offspring, independent of the genetic make-up of the mother plant [24]. This occurs mostly in early stage traits of plant development (e.g. seed mass and germination) but has also been documented for traits over the whole life cycle of the plant (e.g. growth and flowering) [25].

We randomly selected between three and eight seeds from each plant that was collected in the field, and removed the tough outer seed coats by hand. We placed seeds on filter paper in lidded plastic Petri dishes and on the 21 and 22 October 2012, we added MilliQ water to each Petri dish which we then sealed with Parafilm. The seeds began to germinate after a few days. In the weeks that followed, germinating seeds were removed and potted in soil made up with river sand, cocopeat and fertilizer (details in electronic supplementary material, appendix S3, table S3b). Pots were haphazardly placed on the greenhouse benches, and positions were randomly rotated every four to six weeks. Glasshouse temperatures were controlled between 10°C and 25°C and plants were watered every evening at 17.00 by automatic drippers (also at 9.00 in the early stages of establishment). As germination progressed, it became apparent that in some groups more seeds would need to be germinated in order to have enough parent plants. From 5 to 7 December, water was added to a second round of seeds from the South African population. On 14 December, water was added to a second round of seeds from two of the Australian populations (Narooma and Treachery Beach). Seeds from the first round were still germinating at this point and continued to do so up until 14 January.

Plants began to flower in February 2013 and we pollinated them every 3–4 days until senescence in November. For each one of the five populations, we collected pollen from all flowering individuals using a paintbrush and a Petri dish, mixed the pollen and then distributed it back to all available flowers in that population. Flowers that had been pollinated were marked with small tags. In between pollination events, all buds and flowers were covered with small drawstring organza bags. This was both to exclude any possible pollinators, and to collect seeds. In total, we planted 356 parent plants, of which 215 flowered and 186 produced seeds for the next generation of experimental plants.

(d) Experimental plants

We germinated and planted the standardized generation of experimental plants in the same way as the parent plants in the previous year, with water being first added on 3 and 4 December 2013. For South African plants, we randomly selected 15 seeds for germination (if there were less than 15 seeds available we used them all). For Australian plants, we randomly selected 10 seeds per plant for germination. We planted 340 plants for a year-long experiment, and 91 plants for harvesting at 12 weeks to assess above- and below-ground biomass (details in electronic supplementary material, appendix S3, table S3c). After four weeks, we stopped planting seedlings so that the age of all the experimental plants would be within one month of each other. Pot randomization and glasshouse controls were identical to the previous year.

(e) Trait measurements

We marked plants with their week of germination. All plant traits were measured in weekly batches for four consecutive weeks so that all plants in each batch were aged within a week of one another for measurements. Data were collected according to standardized protocols [26].

(i) Plant size and growth form

Because *A. populifolia* is a spreading plant, we measured a range of size traits, including total biomass (a widely accepted indicator of plant performance that is directly related to plant fitness [27]), plant length (an indicator of plant size in spreading plants), height (an indicator of the ability to compete for light) and the ratio of length to height (indicative of the overall shape of the plant). We collected data on plant height and length at nine weeks of age. After this time, the plants required staking and this affected their natural growth form. We measured plant height using a ruler from the base of the stem to the tallest part of the plant. We measured plant length by tracing along the stem with a piece of string and then measuring the string. For growth form, we calculated an index of plant height to length, where plants with an upright growth form would have values closer to one, and plants with a horizontal growth form would have values closer to zero. We measured biomass at 12 weeks by harvesting the subset of plants in the small pots, removing soil, separating above- and below-ground biomass, and drying the material at 60°C for 72 h before weighing. When the plants in the big pots were beginning to senesce (approx. 11 months after planting), we harvested above-ground biomass and dried it at 60°C for 72 h. It was not practical to measure below-ground biomass at this stage.

(ii) Leaf traits

We measured leaf traits when plants were at nine weeks of age. For each plant, we counted down from the top of the plant and removed the leaf and petiole of the second fully formed adult leaf. We immediately measured fresh weight (g) using a Mettler Toledo XS analytical balance, and then scanned an image of the



Figure 2. A representative source plant and a representative introduced plant showing some of the key morphological changes that have evolved since introduction.

leaf using a Canon flatbed scanner (CanoScan LiDE 200). We used IMAGEJ [28] to obtain area and perimeter values. Using a Mercer dial gauge, we took three measurements of leaf thickness (midway between the margin and the midrib at the widest part of the leaf on each side, and then at a similar distance from the top of the leaf, avoiding veins where possible) and then calculated average leaf thickness. We then dried the leaves at 60°C for 48 h and measured dry weight. Using these data, we calculated the following traits: SLA—the one sided area of a fresh leaf divided by its oven-dry mass; leaf dry-matter content (LDMC)—the oven-dry mass of a leaf divided by its water-saturated fresh mass; leaf density—the dry mass of a leaf divided by its volume (thickness × area) and leaf shape (excluding petioles) using a leaf dissection index: (perimeter/[$\sqrt{\text{area}}$]) (following [29]).

Our raw data are available in electronic supplementary material, appendix S6.

(f) Statistical analyses

We compared trait values among the five populations using one-way analyses of variance (ANOVAs, performed in SPSS version 22.0) with a planned contrast between the South African source population and the four Australian introduced populations. Data for SLA were \log_{10} -transformed before analysis. To account for multiple tests (one per trait), we applied a Holm–Bonferroni sequential correction [30]. This did not change the significance of any of these results. Our experimental plants either came from separate maternal lines or were half-sibs. The effect of maternal line made almost no difference to our analyses (electronic supplementary material, appendix S3, table S3d).

Given that the Australian populations experience a range of environments across 5° of latitude, we contrasted all traits among the Australian populations with one-way ANOVAs, followed by a Holm–Bonferroni sequential correction. Only one of the 12 traits showed significant differences among Australian populations (electronic supplementary material, appendix S4, table S4, figure S4).

Our hypotheses were about individual traits, so we began with univariate analyses. However, different plant traits are often correlated [11], so we also used a multivariate analysis of variance (MANOVA) to test for differences between source and introduced plants in the expression of the nine plant traits that were measured on the same individuals. This MANOVA was run using the *manylm()* function of the *mvabund* package in R [31].

3. Results

Introduced *A. populifolia* arrived in Australia less than 100 years ago and yet the plants display striking morphological differences from their South African source population (figure 2).

Consistent with our predictions based on EICA and the higher rainfall in Australia (table 1), introduced plants were

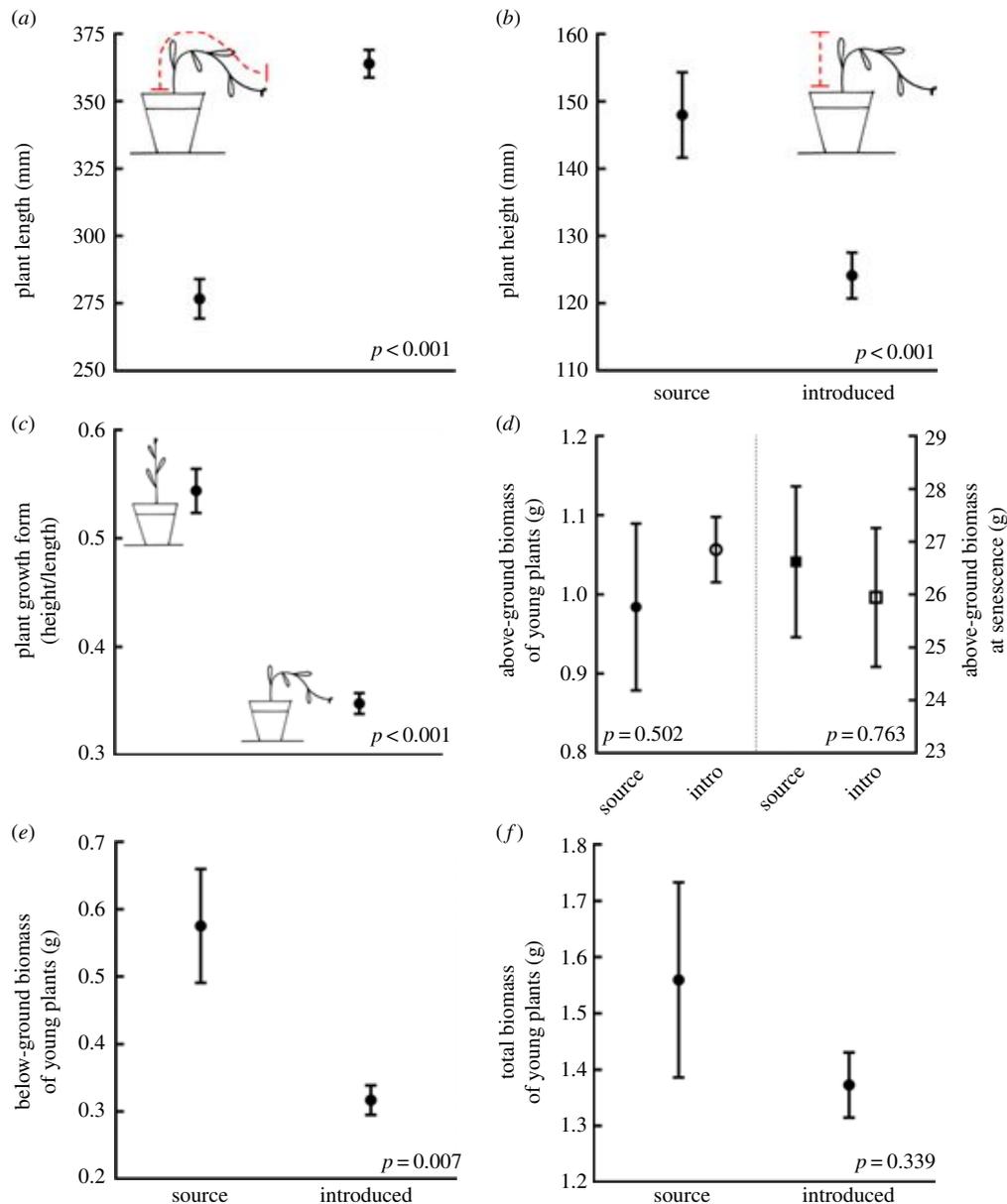


Figure 3. Six plant growth traits comparing source plants and introduced plants showing mean values (\pm s.e.): plant length measured along the stem (a); plant height measured from the base of the stem to the tallest part of the plant (b); plant growth form as an index of plant height to length, where plants with an upright growth form would have values closer to one, and plants with a horizontal growth form would have values closer to zero (c); above-ground biomass of young plants (left-hand side of graph) and of plants at senescence (right-hand side of graph) (d); below-ground biomass (e); and total biomass (f) of young plants. The p -values for each trait are from a planned contrast between the South African source population and the four Australian introduced populations following a one-way ANOVA. y -axes have been truncated. (Online version in colour.)

31% longer than were plants from the source population ($p < 0.001$; figure 3a). However, they were significantly less tall ($p < 0.001$; figure 3b), exhibiting a sprawling, horizontal growth form, as opposed to the more upright source plants ($p < 0.001$; figure 3c). Counter to our predictions, there was no difference in the amount of above-ground biomass produced by introduced and source plants at either the 12-week harvest ($p = 0.502$; figure 3d), or the end-of-life harvest ($p = 0.763$; figure 3d). There was also no significant difference in total biomass between introduced and source plants ($p = 0.339$; figure 3f). However, the introduced plants had 45% less below-ground biomass than did the source

plants ($p = 0.007$; figure 3e), resulting in a higher percentage of overall biomass being allocated above-ground for introduced (78%) compared to source (64%) plants ($p < 0.001$). This result is consistent with our predictions based on rainfall, but counter to the predictions from EICA (table 1).

Contrary to what we predicted based on both EICA and differences in rainfall (table 1), the leaves of the introduced plants were 27% smaller than were the leaves of the source plants ($p < 0.001$; figure 4a). In addition, the source and introduced plants had leaves that were surprisingly different shapes ($p < 0.001$; figure 4b). In the source plants, juvenile leaves had a simple spatulate shape and adult leaves

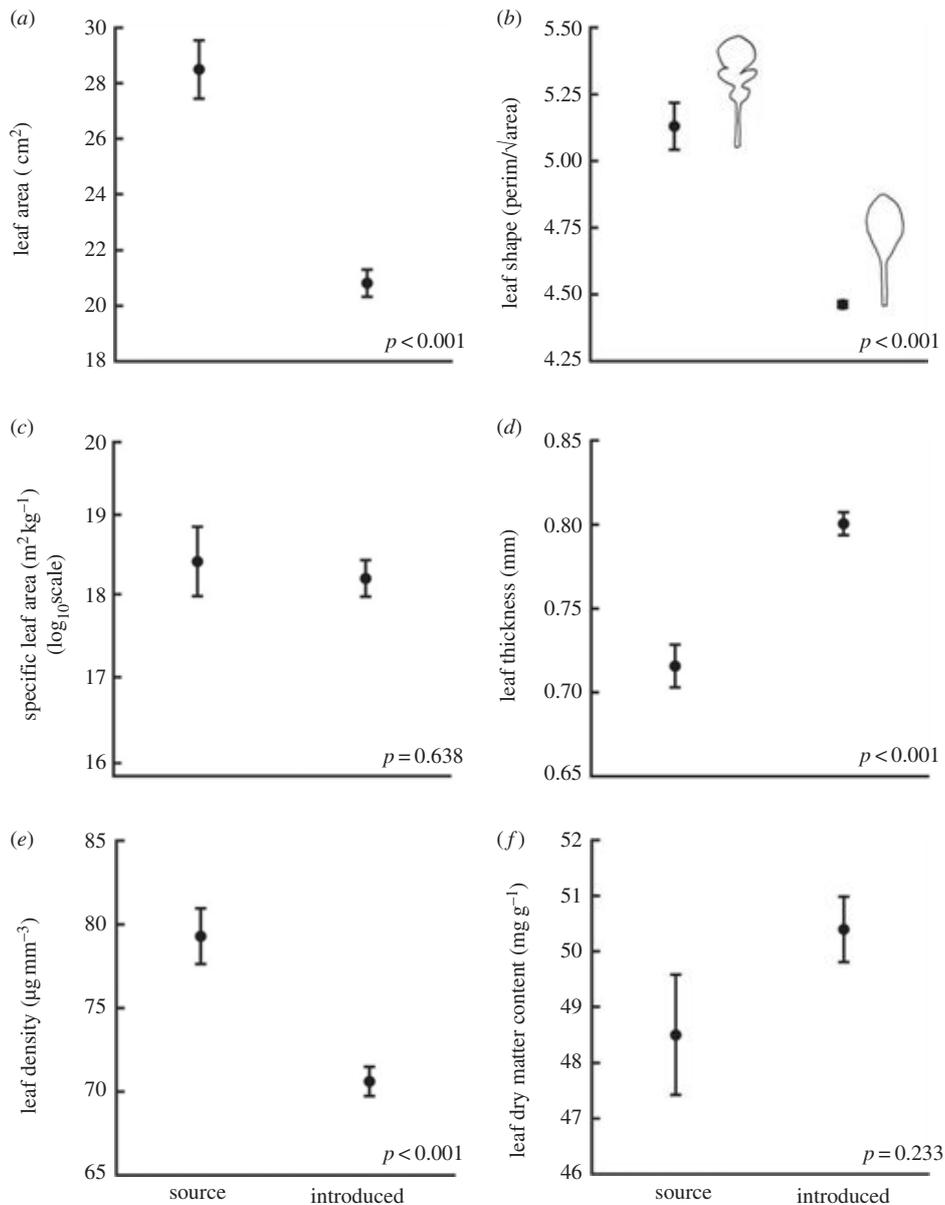


Figure 4. Mean values (\pm s.e.) of six leaf traits comparing source plants and introduced plants. The p -values for each trait are from a planned contrast between the South African source population and the four Australian introduced populations following a one-way ANOVA. y -axes have been truncated.

developed lobes around the perimeter. The introduced plants had lost this lobed adult leaf form and only produced spatulate leaves (figure 5). There was no significant difference between the SLA of the introduced plants and the source plants ($p = 0.638$; figure 4c). SLA is the area of a fresh leaf divided by its dry mass, and in these two groups of plants these traits scale in the same direction—the leaves of the introduced plants had a smaller area ($p < 0.001$) and less dry mass ($p < 0.001$) than did the leaves of the source plants. But leaf thickness and leaf density are also key components of SLA where $SLA \approx 1/(\text{thickness} \times \text{density})$ [32]. Differences in leaf thickness and density do not always affect SLA values because they can trend in opposite directions. For example, tough sclerophyllous leaves (thin and dense) and fleshy succulent leaves (thick and wet) can have the same SLA values [33]. It is crucial to separate



Figure 5. Adult and juvenile leaf pairs of source and introduced plants. Adult leaves are approximately 15 cm in length.

SLA into these two measures, because they can vary separately and are more responsive to environmental gradients than SLA alone [32]. Indeed, in *A. populifolia* the leaves of introduced plants were thicker ($p < 0.001$; figure 4d) and less dense ($p < 0.001$; figure 4e) than were the leaves of the source plants.

There was no significant difference between the LDMC of the introduced plants and the source plants in the planned contrast between all four Australian populations and the South African population ($p = 0.233$; figure 4f). However, LDMC was the one trait for which the Australian populations varied significantly, so we also ran comparisons between each individual Australian population and the population from South Africa. We found a significant ($p = 0.004$) difference in LDMC between the South African population and plants from Malacoota (our southernmost Australian population), but no significant difference between the South African plants and plants from any other Australian population (all $p > 0.5$). Notably, our measured levels of LDMC are some of the lowest reported in the literature (we measured mean LDMC of 50 mg g^{-1} in the introduced range and 49 mg g^{-1} in the source population; in Hodgson [34], the 5–95% range for 1950 species was $93\text{--}387 \text{ mg g}^{-1}$). Since leaf water capacity = $1000 - \text{LDMC}$, this indicates that the leaves of these plants have a very high water-storing capacity.

Source and introduced plants were found to be significantly different from each other when we tested individuals for differences using a MANOVA ($p < 0.001$; electronic supplementary material, appendix S5, figure S5, table S5). Ordination plots (electronic supplementary material, figure S5) and the proportion of variation explained by each trait in the analysis (electronic supplementary material, table S5) are included in appendix S5.

4. Discussion

Introduced populations of *A. populifolia* have evolved many remarkable changes in plant and leaf morphology. These evolutionary changes could have been caused by adaptation to biotic and abiotic factors by natural selection, or by genetic drift.

The introduced plants had evolved to be longer than the source plants. This is consistent with previous work on introduced plants in general [35,36], as well changes observed over time in herbarium specimens of *A. populifolia* [37]. The introduced plants had also evolved a more horizontal growth form than the source plants, which stood more upright. However, even with these differences, there was no difference in the total amount of above-ground biomass produced by introduced and source plants measured at two different life stages. Growing longer but using the same amount of biomass indicates that the introduced plants have evolved cheaper construction costs and a faster growth rate—a strategy consistent with previous studies on other introduced plants [36,38]. In addition, for a coastal dune plant like *A. populifolia*, an increase in growth (especially stem length) and a shift in biomass allocation from below-ground to above-ground are both mechanisms consistent with a response to burial by sand [39]. These strategies could help plants survive burial events [40,41], and might provide an adaptive explanation for the changes we observed.

Contrary to our predictions based on both EICA and the greater rainfall in Australia, the leaves of the introduced plants had evolved to be smaller and thicker than the leaves of the source plants. In general, succulent plants adapt to water-deficient environments by storing water in their leaves, resulting in thick leaves with high leaf water content [33]. Coastal plants like *A. populifolia* must also adapt their leaves to a combination of severe stresses like salt spray, sand burial, wind exposure and nutrient deficiency [42]. Changes to these stresses in the new range could be responsible for driving the evolutionary adaptations we observed in the introduced plants—for example, a decrease in leaf size has been shown with increased wind speed [43] and decreased nutrient availability [44].

Surprisingly, the introduced plants had evolved leaves that were a different shape to the leaves of the source plants (figure 5). The retention of juvenile characteristics into adulthood (known as paedomorphosis) can explain historical evolutionary changes in leaf form [45], but this is the first study showing paedomorphosis occurring in contemporary evolution. This remarkable change in leaf shape could result in a number of functionally significant impacts on thermoregulation and hydraulic efficiency [19]. Previous work shows that the switch between complex and simple leaves could evolve via the regulation of the KNOX (Knotted-like homeobox) genes [46]. A reciprocal genetic transplant experiment on two closely related species with different shaped leaves (*Arabidopsis thaliana*—simple leaves and *Cardamine hirsuta*—complex leaves) showed that when KNOX genes are turned off, a plant that normally produces complex leaves can switch to producing simple leaves [47]. In the case of *A. populifolia* with a single introduction on the east coast of Australia and a presumably small founder population, it is possible that genetic drift could have resulted in certain genetic variants becoming fixed in the introduced plants. One of the fixed changes might have affected regulation of morphological genes such as KNOX to produce the observed switch to simple leaves.

Growing native and introduced plants in a common-environment experiment is a fundamental test for evolutionary changes following introduction to a new range. But without careful consideration of how we sample the native range, the results of these experiments can be distorted. A study re-analysing 32 comparisons of native and introduced populations in common-environment experiments showed that among-population variation due to geographical clines was so substantial that when it was included in the analyses (by including the effect of latitude), it not only changed the significance and magnitude of some trait differences between native and introduced populations but even reversed the direction of some changes [8]. This has worrying implications for how we have been interpreting differences between native and introduced populations in previous common-environment experiments. The only precise test of evolutionary change since introduction can be achieved when we can use the known source population as an accurate point of reference against which we can assess what changes have taken place, as we have done in this study.

In conclusion, we have shown that despite low levels of genetic diversity, introduced species can evolve marked morphological changes. However, these changes are not always consistent with what is predicted by theory. This finding

aligns with a meta-analysis of invasive plants which showed that evolution happens—just not always in the direction predicted by the EICA hypothesis [36], or with predictions based on rainfall. We have also shown how comparing introduced plants with their source population provides a powerful test for uncovering rapid evolution in action.

Data accessibility. Our raw data are available in electronic supplementary material, appendix S6.

Authors' contributions. A.T.M. conceived the idea. A.T.M., W.B.S., R.F. and C.R.B. obtained funding. C.R.B., S.C., P.F. and R.B. collected data. C.R.B. led the analysis and writing with particular input

from W.B.S. and A.T.M. W.B.S. and A.G.B.P. contributed to data analysis. All authors contributed to writing and interpretation.

Competing interests. We have no competing interests to declare.

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References

- Van Kleunen M *et al.* 2015 Global exchange and accumulation of non-native plants. *Nature* **525**, 100. (doi:10.1038/nature14910)
- Vilà M *et al.* 2011 Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecol. Lett.* **14**, 702–708. (doi:10.1111/j.1461-0248.2011.01628.x)
- Vellend M, Harmon LJ, Lockwood JL, Mayfield MM, Hughes AR, Wares JP, Sax DF. 2007 Effects of exotic species on evolutionary diversification. *Trends Ecol. Evol.* **22**, 481–488. (doi:10.1016/j.tree.2007.02.017)
- Cox GW. 2004 *Alien species and evolution: the evolutionary ecology of exotic plants, animals, microbes, and interacting native species*. Washington, DC: Island Press.
- Phillips BL, Brown GP, Webb JK, Shine R. 2006 Invasion and the evolution of speed in toads. *Nature* **439**, 803. (doi:10.1038/439803a)
- Elderkin CL, Klerks PL, Theriot E. 2001 Shifts in allele and genotype frequencies in zebra mussels, *Dreissena polymorpha*, along the latitudinal gradient formed by the Mississippi River. *J. N. Am. Benthol. Soc.* **20**, 595–605. (doi:10.2307/1468090)
- Daehler CC, Strong DR. 1997 Reduced herbivore resistance in introduced smooth cordgrass (*Spartina alterniflora*) after a century of herbivore-free growth. *Oecologia* **110**, 99–108. (doi:10.1007/s004420050138)
- Colautti RI, Maron JL, Barrett SC. 2009 Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. *Evol. Appl.* **2**, 187–199. (doi:10.1111/j.1752-4571.2008.00053.x)
- Dlugosch KM, Parker IM. 2008 Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecol. Lett.* **11**, 701–709. (doi:10.1111/j.1461-0248.2008.01181.x)
- AVH Database. 2016 Australia's Virtual Herbarium, Council of Heads of Australasian Herbaria. See <http://avh.ala.org.au> (accessed on 10 August 2016).
- Moles AT. 2018 Being John Harper: using evolutionary ideas to improve understanding of global patterns in plant traits. *J. Ecol.* **106**, 1–18. (doi:10.1111/1365-2745.12887)
- Wright IJ *et al.* 2004 The world-wide leaf economics spectrum. *Nature* **428**, 821–827. (doi:10.1038/nature02403)
- Australian National Herbarium. 2018 Australian Plant Name Index, version 1.0213. See <http://www.anbg.gov.au/apni/> (accessed 18 December 2018).
- Sakai AK *et al.* 2001 The population biology of invasive species. *Annu. Rev. Ecol. Syst.* **32**, 305–332. (doi:10.1146/annurev.ecolsys.32.081501.114037)
- Rollins LA *et al.* 2013 High genetic diversity is not essential for successful introduction. *Ecol. Evol.* **3**, 4501–4517. (doi:10.1002/ece3.824/pdf)
- Moles AT, Warton DI, Warman L, Swenson NG, Laffan SW, Zanne AE, Pitman A, Hemmings FA, Leishman MR. 2009 Global patterns in plant height. *J. Ecol.* **97**, 923–932. (doi:10.1111/j.1365-2745.2009.01526.x)
- Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012 Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.* **193**, 30–50. (doi:10.1111/j.1469-8137.2011.03952.x)
- Moles AT, Westoby M. 2000 Do small leaves expand faster than large leaves, and do shorter expansion times reduce herbivore damage? *Oikos* **90**, 517–524. (doi:10.1034/j.1600-0706.2000.900310.x)
- Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL, Tsukaya H. 2011 The evolution and functional significance of leaf shape in the angiosperms. *Funct. Plant Biol.* **38**, 535–552. (doi:10.1071/FP11057)
- Blossey B, Nötzold R. 1995 Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *J. Ecol.* **83**, 887–889. (doi:10.2307/2261425)
- Feeny P. 1976 Plant apparency and chemical defense. *Rec. Adv. Phytochem.* **10**, 1–40.
- Coley PD. 1983 Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr.* **53**, 209–229. (doi:10.2307/1942495)
- SANBI database. 2016 South African National Biodiversity Institute. See <http://pza.sanbi.org> (accessed 10 August 2016).
- Roach DA, Wulff RD. 1987 Maternal effects in plants. *Annu. Rev. Ecol. Syst.* **18**, 209–235. (doi:10.1146/annurev.es.18.110187.001233)
- Helenum K, Schaal BA. 1996 Genetic and maternal effects on offspring fitness in *Lupinus texensis* (Fabaceae). *Am. J. Bot.* **83**, 1596–1608. (doi:10.1002/j.1537-2197.1996.tb12818.x)
- Pérez-Harguindeguy N *et al.* 2013 New handbook for standardised measurement of plant functional traits worldwide. *Aust. J. Bot.* **61**, 167–234. (doi:10.1071/BT12225)
- Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E. 2007 Let the concept of trait be functional! *Oikos* **116**, 882–892. (doi:10.1111/j.2007.0030-1299.15559.x)
- Rasband WS. 1997–2016 ImageJ. Bethesda, MD: US National Institutes of Health. See <http://imagej.nih.gov/ij/>.
- McLellan T, Endler JA. 1998 The relative success of some methods for measuring and describing the shape of complex objects. *Syst. Biol.* **47**, 264–281. (doi:10.1080/106351598260914)
- Holm S. 1979 A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**, 65–70.
- Wang Y, Naumann U, Wright ST, Warton DI. 2012 mvabund—an R package for model-based analysis of multivariate abundance data. *Methods Ecol. Evol.* **3**, 471–474. (doi:10.1111/j.2041-210X.2012.00190.x)
- Witkowski ET, Lamont BB. 1991 Leaf specific mass confounds leaf density and thickness. *Oecologia* **88**, 486–493. (doi:10.1007/BF00317710)
- Vendramini F, Díaz S, Gurvich DE, Wilson PJ, Thompson K, Hodgson JG. 2002 Leaf traits as indicators of resource-use strategy in floras with succulent species. *New Phytol.* **154**, 147–157. (doi:10.1046/j.1469-8137.2002.00357.x)
- Hodgson J *et al.* 2011 Is leaf dry matter content a better predictor of soil fertility than specific leaf area? *Ann. Bot.* **108**, 1337–1345. (doi:10.1093/aob/mcr225)
- Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D. 2005 Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* **144**, 1–11. (doi:10.1007/s00442-005-0070-z)
- Felker-Quinn E, Schweitzer JA, Bailey JK. 2013 Meta-analysis reveals evolution in invasive plant species but little support for Evolution of Increased Competitive Ability (EICA). *Ecol. Evol.* **3**, 739–751. (doi:10.1002/ece3.488)

37. Buswell JM, Moles AT, Hartley S. 2011 Is rapid evolution common in introduced plant species? *J. Ecol.* **99**, 214–224. (doi:10.1111/j.1365-2745.2010.01759.x)
38. Kumschick S, Hufbauer RA, Alba C, Blumenthal DM. 2013 Evolution of fast-growing and more resistant phenotypes in introduced common mullein (*Verbascum thapsus*). *J. Ecol.* **101**, 378–387. (doi:10.1111/1365-2745.12044)
39. Gilbert ME, Ripley BS. 2008 Biomass reallocation and the mobilization of leaf resources support dune plant growth after sand burial. *Physiol. Plant.* **134**, 464–472. (doi:10.1111/j.1399-3054.2008.01153.x)
40. Maun MA. 1998 Adaptations of plants to burial in coastal sand dunes. *Can. J. Bot.* **76**, 713–738. (doi:10.1139/cjb-76-5-713)
41. Gilbert ME, Ripley BS. 2010 Resolving the differences in plant burial responses. *Austral. Ecol.* **35**, 53–59. (doi:10.1111/j.1442-9993.2009.02011.x)
42. Hesp PA. 1991 Ecological processes and plant adaptations on coastal dunes. *J. Arid Environ.* **21**, 165–191. (doi:10.1016/S0140-1963(18)30681-5)
43. Retuerto R, Woodward FI. 1993 The influences of increased CO₂ and water supply on growth, biomass allocation and water use efficiency of *Sinapis alba* L. grown under different wind speeds. *Oecologia* **94**, 415–427. (doi:10.1007/bf00317118)
44. Givnish TJ. 1987 Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytol.* **106**, 131–160. (doi:10.1111/j.1469-8137.1987.tb04687.x)
45. Pryer KM, Hearn DJ. 2009 Evolution of leaf form in marsileaceous ferns: evidence for heterochrony. *Evolution* **63**, 498–513. (doi:10.1111/j.1558-5646.2008.00562.x)
46. Bharathan G, Goliber TE, Moore C, Kessler S, Pham T, Sinha NR. 2002 Homologies in leaf form inferred from KNOX1 gene expression during development. *Science* **296**, 1858–1860. (doi:10.1126/science.1070343)
47. Hay A, Tsiantis M. 2006 The genetic basis for differences in leaf form between *Arabidopsis thaliana* and its wild relative *Cardamine hirsuta*. *Nat. Genet.* **38**, 942. (doi:10.1038/ng1835)